This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

Saunders' Question Compends

UC-NRLF
$B 241 030

Essentials of Bacteriology

M. V. Ball, M.D.

Seventh Edition

garis in that the gelatin is less rapidly liquefied. Found also in putrid material.

Proteus Zenkeri (Hauser).—Does not liquefy gelatin; otherwise similar to the other two.

CHAPTER XXI

CHOLERA BACTERIA

Spirillum Choleræ (Koch) (Comma Bacillus of Cholera).—*Synonym, Vibrio Choleræ.*—*Origin.*—Koch, as a member of the German expedition sent to Indi[illegible] in 1883 to study cholera, fo[illegible] micro-organism in the [illegible] contents of cholera pati[illegible] further [illegible]periments i[illegible] with th[illegible] [illegible]ease.

Fig. 64.—Comma ba[illegible] pure culture (× [illegible]

[illegible]orm.—T[illegible] as seen [illegible]arily ap[illegible] short, ar[illegible] [illegible]dy, abo[illegible] size of a [illegible] bacil[illegible] seen [illegible] [illegible]oups, [illegible]rmed, [illegible] [illegible] but [illegible] *a vibrio.* Each [illegible] and possesses a [illegible] straight forms; [illegible] vibrios end to [illegible]

Properties.—[illegible] They are eas[illegible] not been fou[illegible]

Growth.—[illegible] that hav[illegible]

Colon[illegible] points [illegible]

slowly liquefied, a funnel-shaped cavity formed, holding colony in its narrow part, at the bottom, and on the fifth all the gelatin is liquid. If the colonies of three days' gr are placed under microscope, they appear as if compose small bits of frosted glass with sharp, irregular points.

Stab-culture.—After thirty hours a growth can be di guished along the needle-track, and on the surface a cavity is formed, filled by a bubble of air, and this liquefa

lonies after thirty hours (×100) (Fränk Pfeiffer).

n the sixth day, it has reached the
ıg, funnel-shaped, to the bottom of tl
eeks the spirilla are found in little co
of the fluid gelatin. In eight weeks th

ke cultures. A shiny white layer whic

gar.—*Plates* at 37° C. Flat discs, trans

space and at the lowest pos

This edition is not a makeshift revision. The editor and a corps of expert assistants have been working on it for two years. Result—a thoroughly down-to-the-minute dictionary, unequalled for completeness and usefulness by any other medical lexicon published. It meets your needs. It gives you all the *new words*, and in dictionary service *new words* are what you want. Then, it has two-score other features that make it really a *Medical Encyclopedia*.

"I have very carefully looked the volume over, and note with pleasure the many changes in the new edition. It had seemed to me that the work was complete before, but this copy shows how much new matter can be gathered for a great work of this kind."—JOHN B. MURPHY, M. D., *Northwestern University Medical School.*

"Dr. Dorland's Dictionary is admirable. It is so well gotten up and of such convenient size. No errors have been found in my use of it."—HOWARD A. KELLY, *Professor of Gynecology, Johns Hopkins University, Baltimore.*

W. B. SAUNDERS CO., West Washington Square, Phila.

London: 9, Henrietta St

Just Read th) Edition

MEL ARY

W D.,

Boun 0 net;

The b not a revi-
sion of an irely anew
and is con own to be
the most ims to be
complete l the terms
of modern ge vocabu-
lary. Besi k contains
a wealth o s matter is
of particul reparation
for examination.

"I am struck at once with admiration at the compact size and attractive exterior. I can recommend it to our students without reserve."—JAMES W. HOLLAND, M. D., *of Jefferson Medical College.*

"This is a handy pocket dictionary, which is so full and complete that it puts to shame some of the more pretentious volumes."—*Journal of the American Medical Association.*

"We have consulted it for the meaning of many new and rare terms, and have not met with a disappointment. The definitions are exquisitely clear and concise. We have never found so much information in so small a space."—*Dublin Journal of Medical Science.*

"This is a handy little volume that, upon examination, seems fairly to fulfil the promise of its title, and to contain a vast amount of information in a very small space. . . . It is somewhat surprising that it contains so many of the rarer terms used in medicine."—*Bulletin Johns Hopkins Hospital*, Baltimore.

W. B. SAUNDERS CO., West Washington Square, Phila.

London: 9, Henrietta Street, Covent Garden

THE LIBRARY
OF
THE UNIVERSITY
OF CALIFORNIA

PRESENTED BY
PROF. CHARLES A. KOFOID AND
MRS. PRUDENCE W. KOFOID

ESSENTIALS

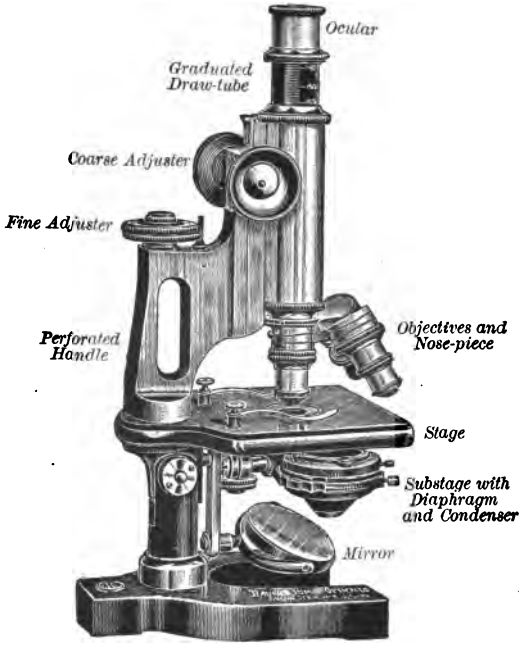
OF

BACTERIOLOGY.

SINCE the issue of the first volume of the **Saunders Question-Compends,**

OVER 342,000 COPIES

of these unrivalled publications have been sold. This enormous sale is indisputable evidence of the value of these self-helps to students and physicians.



BACTERIOLOGIC MICROSCOPE.

SAUNDERS' QUESTION-COMPENDS, No. 20

ESSENTIALS

OF

BACTERIOLOGY

BEING A

CONCISE AND SYSTEMATIC INTRODUCTION TO THE STUDY OF BACTERIA AND ALLIED MICROORGANISMS

BY

M. V. BALL, M.D.

MEMBER OF THE ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA; FORMERLY INSTRUCTOR IN BACTERIOLOGY AT THE PHILADELPHIA POLYCLINIC.

ASSISTED BY

PAUL G. WESTON, M.D.

PATHOLOGIST STATE HOSPITAL FOR INSANE AT WARREN, PA.

SEVENTH EDITION, THOROUGHLY REVISED

With 118 Illustrations, some in Colors

PHILADELPHIA AND LONDON

W. B. SAUNDERS COMPANY

1913

c Mich

Copyright, 1891, by W. B. Saunders. Reprinted October, 1892. Revised, reprinted, and recopyrighted May, 1893. Reprinted June, 1894. Revised, reprinted, and recopyrighted November, 1896. Reprinted October, 1898. Revised, reprinted, and recopyrighted March, 1900. Reprinted May, 1903. Revised, reprinted, and recopyrighted August, 1904. Reprinted October, 1905, and August, 1907. Revised, entirely reset, reprinted, and recopyrighted September, 1908. Reprinted August, 1910. Revised, entirely reset, reprinted, and recopyrighted December, 1913.

Copyright, 1913, by W. B Saunders Company.

PRINTED IN AMERICA

PRESS OF
W. B. SAUNDERS COMPANY
PHILADELPHIA

K-QR46
B232
1913
Biology
Library

PREFACE TO THE SEVENTH EDITION

THIS book has undergone a complete revision and many of the chapters have been rewritten in their entirety. Those which relate to immunity and infection have been carefully edited by Dr. Paul G. Weston, Pathologist at the State Hospital, Warren, Pa., who has also furnished the article on the Wassermann reaction. The author is likewise indebted to him for valuable aid in other portions of the revision.

The author realizes that compends of this nature must necessarily suffer in comparison with the larger and more elaborate works, and he trusts that the reviewers will bear this in mind in their criticisms.

When this book first appeared in 1891 it was one of the first American publications on the subject, and only a few text-books had been issued in other countries. Although since then a great many excellent treatises have appeared, there still remains a place for this compend, and hence this new edition. The author hopes that he has succeeded in incorporating all the newer established facts in bacteriology and in eliminating all that is obsolete and no longer in use.

M. V. BALL.

WARREN, PA., December, 1913.

M345650

PREFACE TO THE FIRST EDITION

FEELING the need of a Compendium on the subject of this work, it has been our aim to produce a concise treatise upon the Practical Bacteriology of *to-day*, chiefly for the medical student, which he may use in his laboratory.

It is the result of experience gained in the Laboratory of the Hygienical Institute, Berlin, under the guidance of Koch and Fränkel; and of information gathered from the original works of other German, as well as of French, bacteriologists.

Theory and obsolete methods have been slightly touched upon. The scope of the work and want of space forbade adequate consideration of them. The exact measurements of bacteria have not been given. The same bacterium varies often much in size, owing to differences in the media, staining, etc.

We have received special help from the following books, which we recommend to students for further reference:

MACÉ: Traité pratique de Bacteriologie.
FRÄNKEL: Grundriss der Bakterienkunde.
EISENBERG: Bakteriologische Diagnostik.
CROOKSHANK, E. M.: Manual of Bacteriology.
GÜNTHER: Einführing in das Studium der Bacteriologie, etc.
WOODHEAD and HARE: Pathological Mycology.
SALMONSEN: Bacteriological Technique (English translation).

M. V. BALL.

CONTENTS

PAGE

INTRODUCTION 17

PART I

GENERAL BACTERIOLOGY

CHAPTER I—STRUCTURE AND DEVELOPMENT OF BACTERIA 21

CHAPTER II—BIOLOGIC AND CHEMIC ACTIVITIES 26

CHAPTER III—INFECTION 30

CHAPTER IV—IMMUNITY 34

CHAPTER V—METHODS OF STUDYING BACTERIA—MICROSCOPE 43

CHAPTER VI—METHODS OF STUDYING BACTERIA (CONTINUED), SOLUTIONS AND FORMULAS FOR STAINING 47

CHAPTER VII—GENERAL METHOD OF STAINING SPECIMENS 54

CHAPTER VIII—SPECIAL METHODS OF STAINING AND MODIFICATIONS 58

CHAPTER IX—CULTIVATION OF BACTERIA 62

CHAPTER X—PREPARATION OF NUTRIENT CULTURE-MEDIA 67

CHAPTER XI—INOCULATION OF CULTURE-MEDIA 78

CHAPTER XII—CULTIVATION OF ANAËROBIC BACTERIA 82

CHAPTER XIII—THE GROWTH AND APPEARANCES OF COLONIES ... 87

CHAPTER XIV—ANIMAL INOCULATION 91

CHAPTER XV—BACTERINS (VACCINES) 95

PART II

SPECIAL BACTERIOLOGY

CHAPTER XVI—SOME COMMON BACTERIA SLIGHTLY PATHOGENIC. 97

Bacterium Prodigiosum 97

Bacillus Mesentericus Vulgatus 98

Bacillus Megaterium 99

Bacillus Ramosus 99

Bacterium Zopfii 100

Bacillus Subtilis (Hay Bacillus) 100

PAGE
Boas-Oppler Bacillus ... 101
Bacillus Violaceus ... 102
Microörganisms Found in Urine ... 102
Micrococcus Ureæ ... 102
Spirilla ... 103
Spirillum Rubrum ... 103
Sarcina ... 103
Sarcina Lutea ... 103
Sarcina Aurantiaca ... 104
Sarcina Ventriculi ... 104

CHAPTER XVII—BACILLUS OF ANTHRAX ... 105

CHAPTER XVIII—BACILLUS TUBERCULOSIS AND ALLIED ORGANISMS ... 110
Other Acid-fast Bacteria ... 116
Bovine and Human Tuberculosis ... 119
Products of Tubercle Bacilli ... 120
Lepra Bacillus ... 122
Smegma Bacillus of Alvarey and Tavel ... 123
Bacillus of Glanders (Bacillus Mallei; Rotz-Bacillus) ... 124

CHAPTER XIX—DIPHTHERIA BACILLUS ... 126

CHAPTER XX—THE COLON-TYPHOID GROUP ... 133
Bacillus Coli ... 134
Bacillus of Typhoid or Enteric Fever ... 135
Antityphoid Bacterins (Vaccines) ... 139
Differentiation Between Colon and Typhoid ... 142
Typhoid Bacilli from Blood ... 143
Paracolon or Paratyphoid Bacilli ... 143
Bacillus Botulinus ... 144
Bacillus Dysenteriæ ... 145
Bacterium Termo ... 147
Bacillus Proteus Vulgaris ... 147
Proteus Mirabilis ... 147
Proteus Zenkeri ... 148

CHAPTER XXI—CHOLERA BACTERIA ... 148
Spirillum Choleræ (Comma Bacillus of Cholera) ... 148

CHAPTER XXII—BACTERIA IN PNEUMONIA ... 155
Diplococcus Pneumoniæ ... 157
Bacillus Pneumoniæ ... 159
Bacillus of Rhinoscleroma ... 159
Bacillus of Influenza ... 160
Koch-Weeks Bacillus ... 161
Bacillus of Pertussis (Whooping-cough) ... 161
Bacillus Melitensis ... 162

CHAPTER XXIII—PYOGENIC COCCI ... 163
Streptococcus Pyogenes; Streptococcus Erysipelatis ... 164
Staphylococcus Pyogenes Aureus ... 166

PAGE
Staphylococcus Pyogenes Albus ... 168
Micrococcus Pyogenes Citreus ... 169
Micrococcus Cereus Albus ... 169
Micrococcus Cereus Flavus ... 169
Micrococcus Pyogenes Tenuis ... 169
Micrococcus Tetragenus ... 170
Morax-Axenfeld Diplobacillus of Conjunctivitis ... 171
Bacillus Pyocyaneus ... 171

CHAPTER XXIV—GONOCOCCUS—MENINGOCOCCUS ... 174
Micrococcus Gonorrhœæ ... 174
Allied Varieties ... 176
Diplococcus Intracellularis Meningitidis ... 177
Bacillus of Soft Chancre, Chancroid ... 178

CHAPTER XXV—ANAËROBIC BACTERIA (BACILLUS OF TETANUS; BACILLUS OF MALIGNANT EDEMA, ETC.) ... 180
Bacillus of Tetanus ... 180
Bacillus Œdematis Maligni; Vibrion Septique ... 184
Bacillus Aërogenes Capsulatus ... 186
Bacillus Enteritidis Sporogenes ... 187
Bacillus Chauvei ... 187

CHAPTER XXVI—HEMORRHAGIC SEPTICEMIA GROUP ... 190
Bacteria of Hemorrhagic Septicemia ... 192
Bacillus of Chicken Cholera ... 193
Bacillus of Erysipelas of Swine ... 194
Bacillus Murisepticus; Mouse Septicemia ... 195
Micrococcus of Mal de Pis ... 196

CHAPTER XXVII—PROTOZOA ... 197
Entamœba Histolytica; Amœba Dysenteriæ ... 198
Life Cycle of Malarial Sporozoa ... 199
Three Forms of Malarial Protozoa ... 201
Methods of Examination for Malarial Organisms ... 203
Trypanosomata ... 204
Trypanosoma Lewisi ... 205
Trypanosoma Brucei ... 206
Sleeping Sickness; Trypanosoma Ugandense Gambiense ... 207
Trypanosoma Evansi ... 208
Herpetomonas (Leishman-Donovan Bodies) ... 208
Piroplasma Bovis (Piroplasma Bigeminum) ... 208
Rabies or Hydrophobia—Negri Bodies ... 209

CHAPTER XXVIII—THE MICRO-ORGANISM OF SYPHILIS AND ALLIED ORGANISMS ... 209
Spirochæta Pallida ... 209
Wassermann Reaction ... 211
Noguchi Modification of Wassermann Reaction ... 214
Luetin Reaction ... 216
Yaws ... 217
Spirillum of Relapsing Fever ... 217
African Tick Fever ... 218

PAGE

CHAPTER XXIX—FILTERABLE ORGANISMS 218
Filterable or Ultra-microscopic Organisms 218
Small-pox and Vaccinia 218
Yellow Fever 219
Measles 219
Typhus Fever 219
Acute Poliomyelitis 219

CHAPTER XXX—YEASTS AND MOLDS 220
Blastomycetes 220
Saccharomyces Cerevisiæ (Torula Cerevisiæ) 220
Saccharomyces Rosaceus; S. Niger; S. Albicans 221
Saccharomyces Mycoderma 221
Oïdium 221
Oïdium Lactis 222
Oïdium Albicans (Soor; Thrush Fungus) 222
Pathogenic Yeasts 222
Blastomycetic Dermatitis or Oïdiomycosis 223
Hyphomycetes (True Molds) 223
Penicillium Glaucum 223
Mucor Mucedo 224
Achorion Schönleinii 224
Trichophyton Tonsurans (Ring-worm) 225
Microsporon Furfur 226
Aspergillus Glaucus 226
Aspergillus Fumigatus 226
Examination of Yeasts and Molds 226
Cladothrices and Streptothrices 227
Crenothrix Kühniana 227
Cladothrix Dichotoma 227
Leptothrix Buccalis 228
Beggiatoa Alba 228
Streptothrix or Cladothrix Actinomyces (Ray Fungus) 228
Streptothrix Maduræ 230
Nocardia (Streptothrix) Farcinica; Bovine Farcin du Bœuf 231
Plant Diseases Due to Bacteria 231

CHAPTER XXXI—EXAMINATION OF AIR, SOIL, AND WATER 232

CHAPTER XXXII—BACTERIA IN MILK AND FOOD 246

CHAPTER XXXIII—BACTERIOLOGIC EXAMINATION OF THE ORGANS AND CAVITIES OF THE HUMAN BODY 257

CHAPTER XXXIV—GERMICIDES, ANTISEPTICS, AND ANTISEPSIS 261

TABLES OF CHIEF CHARACTERISTICS OF PRINCIPAL BACTERIA 268
Non-pathogenic 268
Pathogenic 286

INDEX 305

ESSENTIALS OF BACTERIOLOGY

INTRODUCTION

History.—The microscope was invented about the latter part of the sixteenth century, and soon after, by its aid, minute organisms were found in decomposing substances. Kircher, in 1646, suggested that diseases might be due to similar organisms, but the means at his disposal were insufficient to enable him to prove his theories. Anthony van Leeuwenhoek, of Delft, Holland (1680 to 1723), so improved the instrument that he was enabled thereby to discover micro-organisms in vegetable infusion, saliva, fecal matter, and scrapings from the teeth. He distinguished several varieties, showed them to have the power of locomotion, and compared them in size with various grains of definite measurement. It was a great service that this "Dutch naturalist" rendered the world; and he can rightly be called the "father of microscopy."

Various theories were then formulated by physicians to connect the origin of different diseases with bacteria; but no proofs of the connection could be obtained. Andry, in 1701, called bacteria worms. Müller, of Copenhagen, in 1786, made a classification composed of two main divisions—monas and vibrio; and with the aid of the compound microscope was better able to describe them. Ehrenberg, in 1833, with still better instruments, divided bacteria into four orders: bacterium, vibrio, spirillum, and spirochæte. It was not until

1863 that any positive advance was made in connecting bacteria with disease. Rayer and Davaine had, in 1850, found a rod-shaped bacterium in the blood of animals suffering from splenic fever (*sang de rate*), but they attached no special significance to their discovery until Pasteur made public his grand researches in regard to fermentation and the rôle bacteria played in the economy. Then Davaine resumed his studies, and in 1863 established by experiments the bacterial nature of splenic fever or anthrax.

But the first complete study of a contagious affection was made by Pasteur in 1869, in the diseases affecting silk-worms, —pébrine and flacherie,—which he showed to be due to micro-organisms.

Then Koch, in 1875, described more fully the anthrax bacillus, gave a description of its spores and the properties of the same, and was enabled to cultivate the germ on artificial media; and, to complete the chain of evidence, Pasteur and his pupils supplied the last link by reproducing the same disease in animals by artificial inoculation from pure cultures. The study of the bacterial nature of anthrax has been the basis of our knowledge of all contagious maladies, and most advances have been made first with the bacterium of that disease.

Up to 1875 most medical men believed that bacteria originated in pus and did not associate them with the cause of suppuration. Lister then began the practice of treating wounds and operating antiseptically, having formed the theory that inflammation and suppuration were due to the contamination of wounds by germs from the air, instruments, etc. From 1880 to 1890 the most important organisms were discovered and associated with disease.

In 1890 the discovery of the blood-serum therapy, the antitoxin of Behring, established a new field of research, and much work was undertaken with a view to curing disease.

The researches of Ehrlich and the endeavors of Metchnikoff, Hankin and Ehrlich, to account for the phenomena of immunity, brought forth a great mass of literature and es-

tablished the "lateral-chain" theory and theory of phagocytosis. These theoretic problems occupied the attention of the workers from 1890 to 1905 and are by no means ended. Laveran, in 1881, had discovered the protozoa of malaria, and in 1903 Dutton had associated trypanosomes with sleeping sickness. In 1905 Schaudinn, by demonstrating the cause of syphilis to be a protozoön, gave added importance to this particular group of micro-organisms, and today investigators are looking in this branch of microbiology for the cause of cancer.

The serum reactions of Wassermann and Noguchi, the tuberculins and other products of bacterial growth useful in diagnosis and treatment, have interested the whole medical world, and every physician must of necessity be familiar with some part of this knowledge.

There is hope that the technic and the microscope will receive more attention in the next few years, so that the so-called ultramicroscopic and filterable organisms that are believed to exist will be definitely determined, and also the cause of such epidemic diseases as smallpox and scarlet fever be ascertained.

PART I

GENERAL BACTERIOLOGY

CHAPTER I

STRUCTURE AND DEVELOPMENT OF BACTERIA

THE bacteria occupy the lowest plane of plant life known to us, though they are by no means as primitive in their biology as was formerly supposed, and it is quite possible that still simpler forms may be discovered. The ultra-microscope gives promise of such minute organisms, and has made visible particles of matter $\frac{1}{500}$ the size of our smallest known bacteria.

The numerous unicellular vegetable organisms which form the lower limit of plant life multiply by fission and are

a *b* *c*

Fig. 1.—Types of bacteria: *a*, Micrococcus; *b*, spirillum; *c*, bacillus.

hence called the *Schizophyta*, or splitting plants. This group is subdivided into two classes—(*a*) the *Schizophyceæ*, or fission algæ, and (*b*) the *Schizomycetes*, or fission fungi, or bacteria, as we usually call them.

Bacteria are unicellular masses of protoplasm of microscopic size, multiplying by fission and existing without chlorophyl. Three main types are found: (1) Globular forms, called cocci; (2) straight rod-shaped forms, called bacilli; (3) curved or spiral rods, called spirilla. (See Fig. 1.)

Classifications.—Various ones have been proposed: *Morphologic*, as micrococci, spirilla, and bacilli. *Physiologic*, according to their activities and functions, as acid bacteria, alkali and indol bacteria; then subdivisions, according to motility or need for oxygen, but none are satisfactory. The tendency to place bacteria similar in their disease-producing manifestations in one group is growing, as, for instance, the colon group, the pus-producers, the pneumonic group, etc.

Structure.—Bacteria are cells; they appear as round or cylindric, of an average diameter on transverse section of 0.001 mm. (= 1 micromillimeter), written $\mu = \frac{1}{25000}$ inch. The cell, as other plant-cells, is composed of a membranous cell-wall and cell-contents or cytoplasm.

Cell-wall.—The cell-wall is composed either of hemicellulose, or a form of albumin, since it is less permeable than cellulose membrane. The membrane is firm, and can be brought plainly into view by the action of iodin upon the cell-contents, which contract them.

Cell-contents.—The contents of the cell consist mainly of protoplasm, usually homogeneous, but in some varieties finely granular, or holding pigment, chlorophyl, fat-droplets, and sulphur in its structure. The protoplasm permits osmosis, and is like that of other plant-cells in its structure.

Chemic Composition of Bacteria.—The ash is mostly phosphoric acid; potassium, chlorin, and calcium are present to a small extent; 80 to 90 per cent. is water. The bacteria resemble the lower animals, rather than plants, in chemic composition.

Nuclein, hypoxanthin, and other nitrogen compounds are found in most bacteria. Varies with media in which grown; the proteids are about 10 per cent.; fats, 1 per cent.; ash, 0.75 per cent.

Gelatinous Membrane.—The outer layer of the cell-membrane can absorb water and become gelatinoid, forming either a little envelop or capsule around the bacterium or preventing the separation of the newly branched germs,

forming chains and bunches, as *streptococci* and *staphylococci*. Long filaments are also formed.

Zoöglea.—When this gelatinous membrane is very thick, irregular masses of bacteria will be formed, the whole growth being in one jelly-like lump. This is termed a zoöglea (ζῷον, animal, γλοιός, glue).

Locomotion.—Many bacteria possess the faculty of self-movement, carrying themselves in all manner of ways across the microscopic field—some very quickly, others leisurely.

Vibratory Movements.—Some bacteria vibrate in themselves, appearing to move, but they do not change their

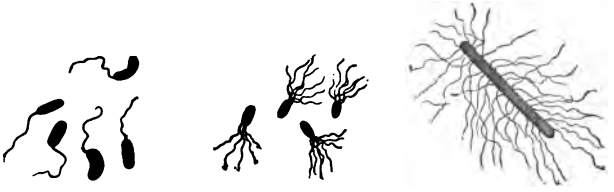


Fig. 2.—Types of flagella: *a*, Vibrio choleræ, one flagellum at the end —monotrichia type; *b*, Bacterium syncyaneum, tuft of flagella at the end, rarely at the side—lophotrichia type; *c*, Bacterium vulgare, flagella arranged all about—peritrichia type (Lehmann and Neumann).

place; these movements are denoted as molecular or "*Brownian*," and are due to purely physical causes, such as may be obtained by suspending fine grains of carmin in water.

Flagella.—Little threads or lashes are found attached to many of the motile bacteria, either at the poles or along the sides—sometimes only one, and on some several, forming a tuft.

These flagella are in constant motion, and can probably be considered as the organs of locomotion; they have not been discovered upon all the motile bacteria, owing, no doubt, to our imperfect methods of observation. They can be stained and have been photographed. (See Fig. 2.) Flagella serve

sometimes to increase food-supply, and have been found on some species which are non-motile.

Reproduction.—Bacteria multiply through simple division or fission, as it is called. Spore formation is simply a resting stage and not a means of multiplication. To accomplish division the cell elongates, and at one portion, usually the middle, the cell-wall indents itself gradually, forming a septum and dividing the cell into two equal parts, just as occurs in the higher plant and animal cells. (See Fig. 3.)

Successive divisions take place, the new members either existing as separate cells or forming part of a community or

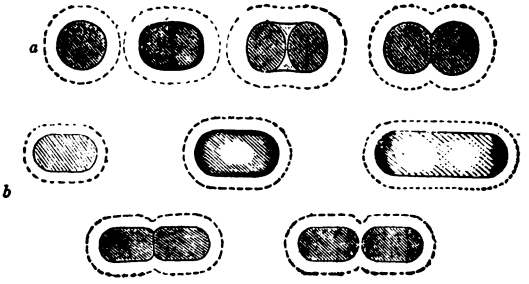


Fig. 3.—Division of bacteria: *a*, Division of a micrococcus; *b*, division of a bacillus (after Macé).

group. It has been computed that if division takes place every hour, as it often does, one individual in twenty-four hours will have 7,000,000 descendants.

Spore Formations.—Two forms of sporulation, *endosporous* and *arthrosporous*.

Endosporous.—First, a small granule develops in the protoplasm of a bacterium; this increases in size, or several little granules coalesce to form an elongated, highly refractive, and clearly defined object, rapidly attaining its real size, and this is the spore. The remainder of the cell-contents has now disappeared, leaving the spore in a dark, very resistant membrane or capsule, and beyond this the weak cell-wall. The

cell-wall dissolves gradually or stretches and allows the spore to be set free.

Each bacterium gives rise to but one spore. It may be at either end or in the middle (Fig. 4). Some rods take on a peculiar shape at the site of the spore, making the rod look like a drum-stick or spindle—clostridium (Fig. 5).

Spore Contents.—What the real contents of spores are is not known. In the mother-cell at the site of the spore little granules have been found which stain differently from the rest

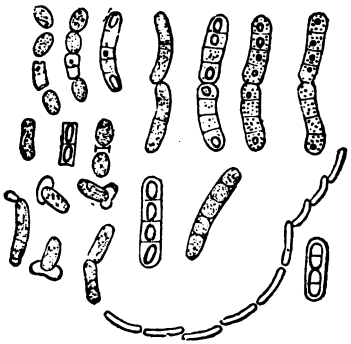


Fig. 4.—Sporulation (after De Bary).

Fig. 5.—Clostridium.

of the cell, and these are supposed to be the beginnings—the *sporogenic bodies*. The most important part of the spore is its *capsule;* to this it owes its resisting properties. It consists of two separate layers—a thin membrane around the cell, and a firm outer gelatinous envelop.

Germination.—When brought into favorable conditions, the spore begins to lose its shining appearance, the outer firm membrane begins to swell, and it now assumes the shape and size of the cell from which it sprang, the capsule having burst, so as to allow the young bacillus to be set free.

Requisites for Spore Formation.—It was formerly thought that when the substratum could no longer maintain

it, or had become infiltrated with detrimental products, the bacterium-cell produced spores, or rather turned itself into a spore to escape annihilation; but we believe now that only when conditions are the most favorable to the well-being of the cell does it produce fruit, just as with every other type of plant or animal life, a certain amount of oxygen and heat being necessary for good spore formation. The question is still unsettled, however.

Asporogenic Bacteria.—Bacteria can be so damaged that they will remain sterile—not produce any spores. This condition can be temporary only or permanent.

Arthrosporous.—In the other group, called arthrospores, individual members of a colony or aggregation leave the same, and become the originators of new colonies, thus assuming the character of spores.

The micrococci furnish examples of this form.

Some authorities have denied the existence of the arthrosporous formation.

Resistance of Spores.—Because of the very tenacious envelop, the spore is not easily influenced by external measures. It is said to be the most resisting object of the organic world.

Chemic and physical agents that easily destroy other life have very little effect upon it.

Many spores require a temperature of 140° C. dry heat for several hours to destroy them. The spores of a variety of potato bacillus (Bacillus mesentericus) can withstand the application of steam at 100° C. for four hours.

CHAPTER II

BIOLOGIC AND CHEMIC ACTIVITIES

Origin of Bacteria.—As Pasteur has shown, all bacteria develop from preëxisting bacteria or the spores of the same. They cannot arise out of nothing.

Distribution.—The wide and almost universal diffusion of bacteria is due to the minuteness of the cells and the

few requirements for their existence. In a drop of water 1,700,000,000 cocci can find space.

Very few places are free from germs; the air on the high seas and on the mountain-tops is said to be free from bacteria, but this is questionable.

Specific Nature.—One kind of bacterium will not produce another kind. A bacillus does not arise from a micrococcus, or the typhoid fever bacillus produce the bacillus of tetanus.

Saprophytes and Parasites.—*Saprophytes:* σαπρός, putrid; φῦτόν, plant. *Parasites:* παρά, aside of; σῖτος, food. Those bacteria which live on the dead remains of organic life are known as saprophytic bacteria, and those which choose the living bodies of their fellow-creatures for their habitat are called parasitic bacteria. Some, however, develop equally well as saprophytes and parasites. They are called *facultative parasites.* All pathogenic (disease-producing) bacteria are parasites.

Conditions of Life and Growth of Bacteria.—*Influence of Temperature.*—In general, a temperature ranging from 10° C. to 40° C. is necessary to the life and growth of bacteria.

Saprophytes take the lower temperatures; parasites, the temperature more nearly approaching the animal heat of the warm blooded. Some forms require a nearly constant heat, growing within very small limits, as the bacillus of tuberculosis.

Some forms can be arrested in their development by a warmer or colder temperature, and then restored to activity by a return to the natural heat.

A few varieties exist only at freezing-point of water, and others again will not live under a temperature of 60° C. and thrive in hot springs at a temperature of 89° C.

For the majority of bacteria a temperature of 60° C. will prevent development, but steam under pressure at 125° C. is necessary to destroy spores. Ice may contain active bacteria; frozen milk permits the growth of bacteria.

Influence of Oxygen.—Two varieties of bacteria in relation

to oxygen—the one *aërobic*, growing in air; the other, *anaërobic*, living without air.

Obligate aërobes, those which exist only when oxygen is present.

Facultative aërobes, those that live best when oxygen is present, but can live without it.

Obligate or *true anaërobes*, those which cannot exist where oxygen is; *facultative anaërobes*, those which exist better where there is no oxygen, but can live in its presence.

Some derive the oxygen which they require out of their nutriment, so that a bacterium may be aërobic and yet not require the presence of free oxygen.

Aërobes may consume the free oxygen of a region and thus allow the anaërobes to develop. By improved methods of culture many varieties of anaërobes have been discovered.

Influence of Light.—Sunlight is very destructive to bacteria. A few hours' exposure to the sun has been fatal to anthrax bacilli and the cultures of Bacillus tuberculosis. The sun's rays, however, must come in direct contact with the germs, and are usually active only on the surface cultures. The rays at the violet end of the spectrum are the most active. The electric arc-light has much the same effect as sunlight on bacteria; the effect of sunlight is not due to heat-rays.

Effects of Electricity.—Electricity arrests growth.

Effects of Röntgen Rays.—Have little or no effect on artificial cultures, but in the living tissues a pronounced bactericidal effect is produced, perhaps through the stimulation of the body-cells.

Moisture.—Water is necessary for the development of most bacteria; complete drying is usually destructive after a few days.

Heat.—Dry heat is much less destructive than moist heat, steam under pressure *most* destructive.

Biologic Activities.—Bacteria feeding upon organic compounds produce chemic changes in them, not only by the withdrawal of certain elements, but also by the excretion of these elements changed by digestion. Sometimes such

changes are destructive to the bacteria themselves, as when lactic and butyric acids are formed in the media.

Oxidation and reduction are carried on by some bacteria. Ammonia, hydrogen sulphid, and trimethylamin are a few of the chemic products produced by bacteria. Nitrites in the soil are reduced to ammonia.

Nitrification.—Albuminoids changed into indol, skatol, leucin, etc.; then these into ammonia, ammonia into nitrites, nitrites into nitrates.

Ptomains.—Brieger found a number of complex alkaloids closely resembling those found in ordinary plants, and which he named ptomains, from πτῶμα, corpse, because obtained from putrefying objects. These were at one time held to be the chief causes of bacterial disease, but are no longer considered of much importance.

Chemic Products.—*Secretions*, as, for instance, enzymes, toxins. *Excretions*, pigments, *indol*, *cell proteins*, *bacterins*.

Proteins.—The *protein contents* of the bacterial cell may cause inflammation and fever.

Producers of Disease.—Various pathologic processes are caused by bacteria, the name given to such diseases being *infectious diseases*, and the germs themselves called disease-producing or *pathogenic bacteria*. Those which do not form any pathologic process are called *non-pathogenic bacteria*.

Fermentation.—This is an important property of bacterial activity.

Enzymes.—An enzyme or ferment is a substance capable of inaugurating a chemic reaction without entering into the reaction, and is a product of living cells.

Bacterial enzymes are closely related to the ferments of special cells of higher animals and plants, like ptyalin and diastase.

Ferments may be *diastatic*, changing starch into sugar, or *proteolytic*, transforming albumins into more soluble substances, of which gelatin liquefaction is an example. *Inverting*, changing a sugar from one that does not undergo fermentation into one that does.

Coagulating, fat-splitting, hydrolytic ferments are some of the other varieties.

Toxins and **toxalbumins** are various albuminoids produced in the animal organism and in culture-media which are very poisonous, and are considered the prime cause of disease.

Putrefaction.—When fermentation is accompanied by development of offensive gases, a decomposition occurs which is called putrefaction, and this, in organic substances, is due entirely to bacteria.

Pigmentation.—Some bacteria are endowed with the property of forming pigments either in themselves, or producing a chromogenic body which, when set free, gives rise to the pigment. In some cases the pigments have been isolated and many of the properties of the anilin dyes discovered in them.

Phosphorescence.—Many bacteria have the power to form light, giving to various objects which they inhabit a characteristic glow or phosphorescence.

Fluorescence.—An iridescence, or play of colors, develops in some of the bacterial cultures.

Gas-formation.—Many bacteria, anaërobic ones especially, produce gases, noxious and odorless; in the culture-media the bubbles which arise soon displace the media.

Odors.—Some germs form odors characteristic of them: some are pleasant and even fragrant; others, foul and nauseous.

Effect of Age.—With age, bacteria lose their strength and die.

CHAPTER III

INFECTION

How Bacteria Cause Disease.—Many theories have been advanced to explain the action of bacteria in causing disease, but only a few of the more important ones can be discussed. Nearly all the changes found in the organs of the

body are similar to those produced by drugs and can be reproduced by the injection of bacterial poisons.

Infection is the successful invasion of an organism by microparasites, and implies an abnormal state resulting from the deleterious action of the parasite upon the host.

Sources of infection may be exogenous or endogenous. *Exogenous infections* result from the successful invasion of the body by microparasites from sources entirely apart from the individual infected. Infection by the typhoid bacillus from water or milk, by the Spirochæta pallidum from dental instruments or drinking-cups, contraction of smallpox from fomites, and contraction of malaria from the bites of mosquitos are examples of exogenous infection.

Endogenous infections result from the successful invasion of the body by microörganisms normally present on the body. The skin and mucous membranes furnish lodgment for a great variety of virulent pathogenic organisms which, when the resistance of the body is lowered, immediately become invasive. The pneumococcus is a normal inhabitant of the mouth and pharynx, but causes no infection until the body resistance is lowered. When this occurs, tonsillitis, pharyngitis, or lobar pneumonia may follow.

Pathogenesis.—The ability of a microörganism to do harm depends on its invasive powers and its ability to generate toxins or both.

Toxins.—Little is known of the chemic nature of toxins. Undoubtedly some are related to albumins. Others give no reactions common to compounds of this group.

(A) *Intracellular or Insoluble Toxins.*—These are chiefly within the bodies of the bacteria, and are set free by disintegration of the organism. This group comprises most of the pathogenic bacteria.

(B) *Extracellular or Soluble Toxins.*—These toxins are apparently excreted by the bacteria, and are found in the surrounding medium. This group includes the diphtheria and tetanus bacilli.

It has been shown that bacteria which apparently do not

produce toxins in artificial media may do so in the human body. These toxic substances are formed by the bacteria to combat the body defenses, and have been called by Bail *aggressins*. They have a paralytic action on phagocytes. A sublethal dose of bacteria, if injected along with aggressin, will cause death.

Toxins are not stable, though tetanus toxin has been kept in powdered form for a number of years. They are soluble in water, destroyed by heat (thermolabile), and precipitated by ammonium sulphate.

The Cardinal Conditions for Infection.—(1) *The microörganism must be sufficiently virulent;* (2) *it must enter in sufficient numbers and by appropriate channels; and* (3) *the host must be susceptible.*

Virulence is a very variable quality, and depends on the ability of the micro-organism to invade or produce toxin or both. *The virulence may be decreased* by repeated transplanting on artificial culture-media or by the action of heat. *It may be increased* by adding animal juices to the culture-medium, by inclosing the micro-organism in a collodion sac, and placing the sac in the abdominal cavity of an animal, and by repeatedly passing it through animals.

Infection Depends on Quantity of Bacteria.—Unless a sufficient number of bacteria enter the tissues no infection follows, because the body defenses immediately destroy the bacteria. The number necessary to cause infection depends on their virulence and the susceptibility of the host. Streptococci may become so virulent that a single coccus will cause death in a rabbit. It has been found that 820 tubercle bacilli are necessary to kill a guinea-pig, and 1,000,000 staphylococci to kill a rabbit. The period of incubation can be explained on the supposition that the organism requires a definite time to generate the amount of toxin necessary to produce symptoms.

Avenues of Infection.—The organism *must gain entrance into the tissue or find lodgment on some part of the body that has been injured.* Even when several avenues of infec-

tion are open, the parasite most commonly invades through one that may, therefore, be regarded as the most appropriate for entrance; this channel furnishes the typical picture of the infection.

Susceptibility of the Host.—Susceptibility varies in different species of animals, in different members of the same species, in the same individual at different times, and in the same individual to different organisms.

Susceptibility may be natural, as in smallpox; *acquired*, as from exposure to conditions which lower the vitality, such as hunger, cold, intoxication, fatigue, inhalation of noxious vapors, and traumatic shock. *Inherited susceptibility* also occurs. The transmission of certain inherited characteristics, as narrow chest, predisposes to infection of the lungs.

Mixed infections are the result of two or more microorganisms successfully invading and intoxicating the host at the same time.

Local Effects of Bacteria.—By *mechanical obstruction* from rapid growth of the bacteria, thrombosis, with its consequences, may occur. *Destruction of a part* of the cells of a tissue with necrosis can arise from irritation, the bacteria acting as a foreign body.

General Effects.—*Bacteremia* or *septicemia* occurs when bacteria proliferate and enter the whole system, as when *anthrax* and *typhoid* cause general disease.

Toxemia.—When the poisons become widely distributed, though the bacteria remain few and localized, and never or seldom enter the circulation, as diphtheria and tetanus.

Pyemia, a form of bacteremia, in which secondary or metastatic foci of suppuration occur throughout the body.

Suppurative bacteria are those which give rise to inflammation and suppuration locally at the point of entrance, and secondarily through metastasis. Any organism may cause suppuration, but certain ones are peculiarly inclined to give rise to pus, and are known as *pyogenic* organisms.

Specific Bacteria.—*Infective bacteria* are, as a rule, *specific*, the particular toxin having a specific action and caus-

ing a disease peculiar to the micro-organism. Thus typhoid fever is a disease distinctly different from tuberculosis; the infective organisms are distinct and the poisons they produce have specific characteristics.

The Nature of Toxins.—Very similar to the venom of serpents; highly poisonous in minute doses ($\frac{1}{1000}$ gram of tetanus toxin will kill a horse weighing 600 kilos—1200 pounds). At first toxins were called *ptomains*, or cadaveric alkaloids; but this term is applied now to such poisons as have a *basic* nature and arise in decomposing meat, cheese, and cream as a result of chemical change in the material, the bacteria causing the change. Then they were called *toxalbumins*, and were supposed to belong to an albumin series; but when the bacteria are grown in non-albuminous media, the toxins correspond more in their chemical composition to a *ferment*, and therefore it is supposed that the albumin part of the toxin is furnished by the blood or albuminous media in which it is formed. The term *toxin* is to be preferred in speaking of bacterial poisons.

CHAPTER IV

IMMUNITY

Ordinary Defenses to Bacterial Invasion.—The unbroken skin and the connective tissue underneath prevent the passage of bacteria. The unbroken mucous surface of eye, nose, and mouth, because of the continuous washing, prevents the numerous bacteria that are constantly present in the discharge from finding suitable lodgment. The hairs and ciliated epithelium in upper respiratory tract retain many a dust particle and pathogenic cell on its way to the lungs. The acid gastric juice is destructive to most bacteria, and protects not only the stomach, but the intestines as well.

The intestinal secretions are but mildly preventive of bacterial growth, but peristalsis aids in dislodgment of microorganisms.

Immunity is the ability to resist infection and intoxication. It is always relative and never absolute.

Immunity { Natural; Acquired { Active. Passive.

Natural immunity is a natural inherited resistance against infection or intoxication, peculiar to certain groups of animals, but common to all the individuals of these groups. It is peculiar to the kind of animal, not to the individual. Thus the field mouse is susceptible to glanders; the house mouse is slightly immune, and the white mouse is immune.

Acquired immunity is resistance to infection or intoxication possessed by certain animals of a naturally susceptible kind, in consequence of circumstances peculiar to them as individuals. *Active acquired immunity* arises from the activities performed by the organism itself. It depends on infection or intoxication, which may have been *accidental or intentional; i. e.*, for the purpose of producing immunity. Some accidental infections, recovery from which renders the individual immune, are measles, scarlet fever, and smallpox. Other infections are followed by an immunity of short duration, as typhoid fever and pneumonia.

Immunity from intentional infection or intoxication is produced by—(*A*) *bringing about a different disease*, as in the production of vaccinia to bring about immunity to smallpox. (*B*) *Inoculation with killed bacteria*, as in the protective inoculation against typhoid fever or bubonic plague. (*C*) *Inoculation with bacterial products*, as diphtheria or tetanus toxin. (*D*) *Inoculation with attenuated cultures* of microorganisms, as in Pasteur's anthrax vaccine or Haffkine's cholera vaccine. (*E*) *Inoculation with virus of increasing virulence*, as in the protective inoculations against hydrophobia.

(*F*) *Inoculation with sublethal doses of virulent bacteria*, beginning with small doses, and gradually increasing their size. Guinea-pigs inoculated in this way have acquired a marked degree of immunity to tuberculosis.

Passive acquired immunity is always artificially supplied to the animal. It follows when antibodies are supplied from an immunized animal to one normally susceptible. Immunization against diphtheria by the injection of diphtheria antitoxin is a good example.

Theories of Immunity.—*Phagocytic Theory of Metchnikoff.*—Immunity is dependent on the action of the phagocytes and their ferments. The *phagocytes* are of two kinds —*macrophages*, which include endothelia and connective-tissue cells, and *microphages*, the polymorphonuclear leukocytes. These phagocytes liberate ferments—macrocytase and microcytase respectively. Infecting organisms and their toxins are destroyed by the phagocytes and their ferments. This theory has been replaced by the *lateral or side-chain theory of Ehrlich.*

Ehrlich's Lateral Chain Theory.—This derives its name from the fact that it presents an analogy to what happens in the benzol ring of organic chemistry when its replaceable atoms of hydrogen are substituted by "side chains" of more or less complex nature. The molecule of protoplasm is supposed to consist of a central atom group, provided with a large number of side chains which subserve the vital processes of the molecule by combining with other organic molecules. These side chains are called *receptors*, and are of many different kinds, so as to fit them for combination with many different varieties of extraneous groups.

Three orders of receptors are described: *Receptors of the first order*, which concern themselves with the assimilation of simple substances (toxins, ferments, and other cell secretions), utilizing a single haptophore. *Antitoxins*, as an example.

Receptors of the second order, which, in addition to the haptophore group, possess a second group, which affects the

coagulation. *Toxins* may be regarded as receptors of the second order thrust off by the bacteria.

Receptors of the third order, which possess two haptophore groups, one of which effects the union with the food-stuffs, whereas the other lays hold on certain substances circulating in the blood plasma, the complements, which cause ferment-like actions—*cytolysins*, as an example.

The Formation of Antitoxin According to the Lateral Chain Theory.—The toxin molecule consists of two groups: (*A*)

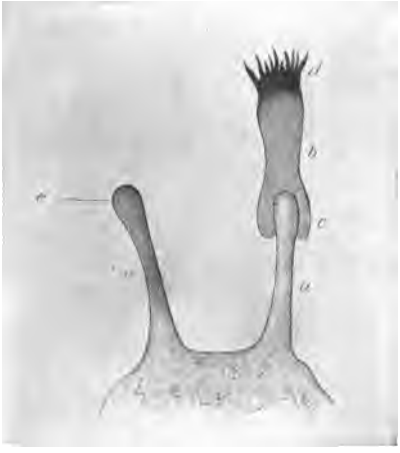


Fig. 6.—Graphic representation of receptors of the first order and of toxin uniting with the cell-receptor: *a*, Cell-receptor; *b*, toxin molecule; *c*, haptophore of toxin molecule; *d*, toxophore of toxin molecule; *e*, haptophore of the cell-receptor (Ehrlich).

The haptophore or combining group, by which the toxin molecule can join the receptor of the cell, and (*B*) the *toxophore*, or poisoning group, by which means it can attack the cell protoplasm after having been fixed to it by the haptophore group.

The effect of the toxin depends on the number of molecules attached to the cell. A great number would bring

about death of the cell, while a few would act as an irritant.

Weigert's Law.—When a cell is attacked by a few molecules of toxin, it reacts by forming new side chains or receptors, and, in accordance with the law of Weigert, always in excess. Repeated injections of toxins in increasing doses cause such an overproduction of receptors of the first order that they are thrust from the cell and float free in the blood-

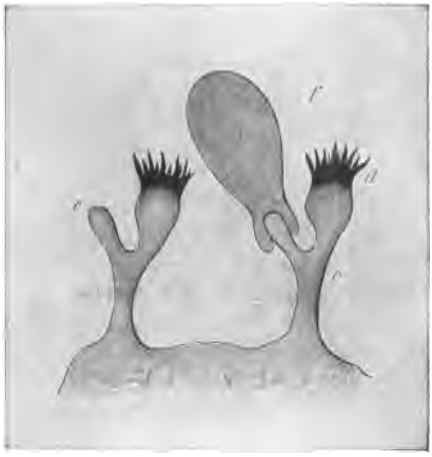


Fig. 7.—Graphic representation of receptors of the second order and of some substances uniting with one of them: *c*, Cell-receptor of the second order; *d*, toxophore or zymophorous group of the receptor; *e*, haptophore of the receptor; *f*, food substance or product of bacterial disintegration uniting with the haptophore of the cell-receptor (Ehrlich).

stream. Here they can combine with toxin molecules, just as when they are attached to the cell. By thus combining, they prevent the toxin from reaching the cells.

Antitoxins are specific in their action; that is, each antitoxin will neutralize only a certain toxin. Thus diphtheria antitoxin will not neutralize tetanus toxin or snake venom,

nor will tetanus antitoxin neutralize diphtheria toxin or snake venom.

Lock and Key Theory.—This specific action is explained by supposing the molecule of toxin to have a shape peculiar to itself. The molecule of diphtheria toxin is of such shape that the haptophore end will fit only on certain receptors of a cell; the molecule of tetanus toxin will fit on only certain other receptors.

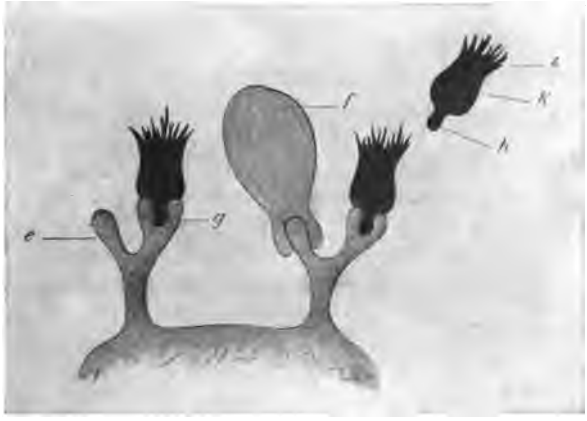


Fig. 8.—Graphic representation of receptors of the third order, and of some substance uniting with one of them: *c*, Cell-receptor of the third order, amboceptor; *e*, one of the haptophores of the amboceptor with which some food substance or product of bacterial disintegration, *f*, may unite; *g*, the other haptophore of the amboceptor with which complement may unite; *k*, complement; *h*, the haptophore, and *z*, the zymotoxic group of the complement (Ehrlich).

An *antitoxic serum* is a suspension of receptors of the first order in blood-serum. Antitoxins for diphtheria and tetanus are the most common.

Precipitins are bodies in serum which, when added to a protein in solution, will cause a precipitate to form. The *precipitins are specific* and act only with similar proteins.

When a protein or food substance is injected into an

animal and becomes attached to the cell receptors of the second order by means of its haptophore group, the cell is irritated and new receptors are formed. Further injection of larger amounts of protein stimulate the cell to such an excessive formation of these receptors that they are thrust free into the blood-stream.

A *precipitin serum* is a suspension of receptors of the second order in blood-serum.

The phenomenon of precipitation has found forensic application in the identification of blood-stains.

Agglutinins are bodies present in a serum which, when added to bacterial cells, cause them to clump, and, if motile, to lose their motility. They are specific when diluted, and of value in diagnosis in such diseases as typhoid and Malta fever.

Agglutinins are formed in response to the stimulus given the cells of a body by the union of antigenic cell-receptors with receptors of the second order of the cells of the animal receiving the injection. Repeated injections stimulate the cells to the formation of such excessive quantities of these receptors that they are thrown from the cells into the blood-stream.

Agglutinins bear no relation to the degree of immunity, and should never be used as an index to immunity.

Cytolysins are bodies present in a serum which will dissolve or destroy cells (corpuscles, bacteria, etc.).

They are formed in the same manner as the agglutinins, except that *receptors of the third order* are involved. Receptors of the third order have a double combining affinity. One part attaches itself to the receptor of the cell injected, and the other combines with complement.

Complement (*alexin* or *cytase*) is a thermolabile, ferment-like body found in all normal sera.

Amboceptors, "*substance sensibilatrice*," *fixateur*, *copula*, and *desmon* are names given to receptors of the third order.

Cytolytic sera are of little use in medicine. Sera have been prepared against staphylococci, pneumococci, streptococci,

and others. Wassermann has made a very efficacious anti-meningococcus serum.

Opsonins.—Opsonins are substances in the blood-serum which act on bacteria and prepare them for phagocytosis. Opsonins can be increased by whatever increases immunity. An increase is coincident with increased immunity. The most common method of bringing about an increase is by the injection of killed cultures of bacteria.

Opsonins normally present in the serum are not specific. Opsonins resulting from reaction to infection or inoculation are specific.

The **opsonic index** is the ratio between the number of bacteria ingested by living leukocytes when operating in the serum of a test and in normal serum respectively.

After the injection of bacteria the opsonic index falls for a short time. This period is called the *negative phase*, and is followed by a rise in the index—the *positive phase*.

The "estimation of the opsonic index is a very complicated way of finding out very little," and has been abandoned by the great majority of workers.

Antigens.—Any substance that has, when injected into the body, power to produce an antitoxin or antibacterial body is called an *antigen*.

The toxin of diphtheria, if injected, stimulates the normal cells to produce chemic substances (free receptors) which are at liberty to attach themselves to the active toxin molecules and thus save the body cells from being acted upon; toxin is, therefore, an *antigen*.

Substances which have the power of destroying bacteria are called *bactericides;* those which dissolve them merely are called *bacteriolysins*.

Hemolysis.—When the hemoglobin of the red blood-cells is liberated, hemolysis is said to occur. This is brought about by the injection of certain substances, or *hemolysins;* these are present normally in some sera, and can be developed in others. Lysins and bactericidal substances seem to have two parts—one destroyed by heat (thermolabile), called

complement (the completor), and one, more resistant, called the *amboceptor*, or combinor, which unites with complement and with the cell. For *lysis*, therefore, it is necessary that ambocepter be united to the bacteria or cell, and that complement be present or added to join with amboceptor, completing the circuit. *Complement* may be prevented from combining with amboceptor by "*deviation of the complement.*" The amboceptor may be in excess, and the free group absorb or attach itself to all the available complement, leaving none to join the amboceptor; or anti-complements may be present to monopolize all this complement and leave none free to unite with amboceptor. This deviation prevents lysis.

Fixation of Complement.—By adding a definite standardized complement to a mixture of antigen and amboceptor of a similar kind the complement is bound or fixed, and none is left free. If the amboceptor is not like the antigen, the complement will not unite the two, will not be bound, and is free to unite with any other amboceptor that may be introduced. If this be a hemolytic amboceptor, and red corpuscles are added as an indicator, the cells will lose their hemoglobin, because hemolysis will occur from the completing of the reaction. The complement will unite to hemolytic amboceptor, since it is not fixed or bound by the other amboceptor, and the other amboceptor is not of the same nature as the antigen. This is the principle of the *Wassermann serum reaction* or *test.*

Anaphylaxis or Allergy.—Under certain circumstances the second injection of a proteid as antigen instead of rendering immune, produces *hypersensitiveness.* Behring, in 1892, noticed this with injections of antitoxin, and called it "*hypersusceptibility.*"

Richet, in 1904, called a similar condition *anaphylaxis*, or the reverse of prophylaxis, and von Pirquet introduced the term "*allergy*," "*altered reactivity*," to express the same thing. Guinea-pigs may be rendered so sensitive by 0.001 c.c. of horse-serum that a second dose within a week or a few days produces fatal shock.

Other proteins, like beef-serum, egg-albumin, red blood-corpuscles, have produced similar results, varying doses and periods of incubation. Human beings may be sensitized by single injections of horse-serum.

Hay-fever, asthma, puerperal convulsions, and sympathetic ophthalmia partake of the nature of anaphylactic reactions, and the peculiar intolerances to certain articles of food may be better explained by the same theory.

The sudden attacks of collapse and death which have followed the injection of even small doses of antitoxins made from horse-serum are believed to come from this condition of hypersensitiveness.

The use of globulins instead of the entire serum has lessened the danger from anaphylaxis.

CHAPTER V

METHODS OF STUDYING BACTERIA.—MICROSCOPE

Microscope.—Most clinical instruments now on the market have all the necessary appliances for bacterial examination. Three objectives are advisable—16 mm. (⅔ inch); 4 mm. (1/6 inch); 2 mm. ($\frac{1}{12}$ inch). It is not so much required to have a picture very large, as to have it sharp and clear.

Oil-immersion Lens.—The penetration and clearness of a lens are very much influenced by the absorption of the rays of light emerging from the picture. In the ordinary dry system many of the light rays, being bent outward by the air which is between the object and the lens, do not enter the lens, and are lost. By interposing an agent which has the same refractive index as glass, *cedar-oil* or *clove-oil*, for example, all the rays of light from the object enter directly into the lens.

The "homogeneous system," or oil-immersion lens, con-

sists of a system of lenses which can be dipped into a drop of cedar-oil placed upon the cover-glass, and which is then ready for use.

Abbé's Condenser.—The second necessary adjunct is a combination of lenses placed underneath the stage, for bringing wide rays of light directly under the object. It serves to intensify the colored pictures by absorbing or hiding the unstained structure.

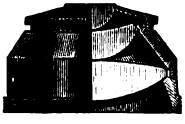


Fig. 9.—Abbé's condenser.

This is very useful in searching a specimen for bacteria, since it clears the field of everything that is not stained. It is called Abbé's condenser (Fig. 9). Together with it is usually found an instrument for shutting off part of the light—a *blender* or diaphragm (Fig. 10). When the bacteria have been found, and their relation to the structure is to be studied, the "Abbé" is generally shut out by the iris blender, and the structure comes more plainly into view. A white light (daylight or a

Fig. 10.—Iris blender.

Welsbach burner) is best for bacterial study: use the plane mirror with the condenser.

For all *stained bacteria* the oil-immersion lens and Abbé condenser, without the use of blender. For *unstained specimens*, oil-immersion and the narrowed blender.

When examining with low-power objective, use a *strong*

ocular. When using high-power objective use *weak* ocular. A *revolving* nose-piece will be found very useful, since it is sometimes necessary to change the objective on the same field, and this insures a great steadiness of the object.

Great cleanliness is needed in all bacteriologic methods, but nowhere more so than in the microscopic examination.

The cover-glass should be very carefully washed in alcohol, and dried with a soft linen rag. To remove the stains on the cover-glasses that have been used they should be soaked in hydrochloric acid or placed in a 6 per cent. aqueous solution of potassium dichromate with 6 per cent. of strong sulphuric acid, washed in water, and kept in absolute alcohol.

Examination of Unstained Bacteria.—As the coloring of bacteria kills them and changes their shape to some extent, it

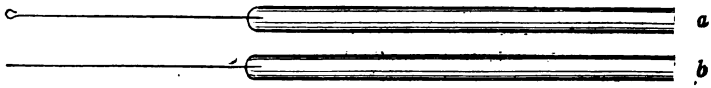


Fig. 11.—Platinum needles for transferring bacteria, made from No. 27 platinum wire inserted in glass rods: *a*, Looped needle; *b*, straight-pointed needle (McFarland).

is preferable to examine bacteria, when possible, in their natural state.

We obtain the bacteria for examination either from liquid or solid media.

From Liquids.—With a long platinum needle the end of which is bent into a loop (Fig. 11, *a*) obtain a small drop from the liquid containing the bacteria, and place it on a cover-glass or slide, careful that no bubbles remain.

Sterilize Instruments.—Right here we might say that it is best to accustom one's self to pass all instruments, needles, etc., through the flame before and after each procedure; it insures safety; and once in the habit, it will be done automatically.

From Solid Media.—With a straight-pointed platinum needle (Fig. 11, *b*) a small speck of the medium is taken and

rubbed upon a glass slide with a drop of sterilized water or bouillon, and from this a little is taken on cover-glass, as before.

The cover-glass with its drop is now placed on the glass slide, carefully pressing out all bubbles. Then a drop of cedar-oil is laid on top of the cover-glass, and the oil-immersion lens dipped gently down into it as close as possible to the cover-glass, the narrow blender *shutting off* the Abbé condenser, for this being an unstained specimen, we want but *little light.* We now apply the eye, and if not in focus, use the fine adjustment or the coarse, but always *away from the object—i. e.,* toward us—since the distance between the speci-

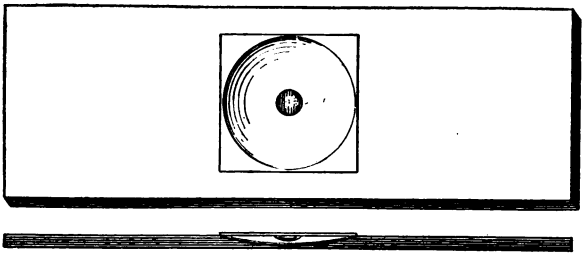


Fig. 12.—A "concave slide" with "hanging drop" (McFarland).

men and the lens is very slight, it does not require much turning to break the cover-glass and ruin the specimen. Having found the bacterium, we see whether it is bacillus, micrococcus, or spirillum, discover if it is motile or not. The phenomenon of agglutination is observed in this way.

Hanging Drop (Fig. 12).—When the looped platinum needle is dipped into a liquid, a very finely formed globule will hang to it; this can be brought into a little cupped glass slide (an ordinary microscopic glass slide with a circular depression in the center) in the following manner: The drop is first brought upon a cover-glass; the edges of the concavity on the glass slide are smeared with vaselin, and the slide inverted over the drop; the cover-glass sticks to the smeared slide,

which, when turned over, holds the drop in the depression covered by the cover-glass, thus forming an air-tight cell; here the drop cannot evaporate. Both slide and cover-glass should first be sterilized by heat.

Search for the bacteria with a weak lens; having found them, place a drop of cedar-oil upon the cover-glass, and bring the oil immersion into place (here is where a nose-piece comes in very useful), careful not to press against the cell, for the cover-glasses are very fragile in this position.

Search the *edges* of the drop rather than the middle; the bacteria will usually be very thick in the center and not so easily distinguished.

Spores, automatic movements, fission, and cultivation in general can be studied for several days. This *moist chamber* can be placed in a brood-oven or on the ordinary warming stage attachment of the microscope.

Hanging Block.—A small slice of agar containing some of the growth seared to the glass slide with a hot needle.

Agglutination as observed in Widal's test is best seen in the hanging drop.

CHAPTER VI

METHODS OF STUDYING BACTERIA (Continued).—SOLUTIONS AND FORMULAS FOR STAINING

STAINING or coloring bacteria is done in order to make them prominent and to obtain permanent specimens. It is also necessary to bring out the structure of the bacteria, and serves in many instances as a means of diagnosis; it would be well-nigh impossible to discover them in the tissues without staining.

Anilin Colors.—Of the numerous dyes in the market, nearly all have, at one time or other, been used in staining

bacteria. But now only a very few find general use, and with methylene-blue and fuchsin nearly every object can be accomplished.

Basic and Acid Dyes.—Ehrlich was the first to divide the anilin dyes into two groups, the basic colors to which belong—

Gentian-violet, or pyoktanin.	Basic fuchsin.
Methyl-violet, or dahlia.	Bismarck-brown
Methylene-blue (*not* methyl blue).	Thionin.
	Safranin.

And the acid colors to which eosin and acid fuchsin belong.

The *basic aniline* dyes stain the bacteria and the nuclei of cells; the *acid* dyes stain chiefly the tissue, leaving the bacteria almost untouched. *Carmin* and *hematoxylin* are also useful as contrast stains, affecting bacteria very slightly. The anilin dyes are soluble in alcohol or water or a mixture of the two.

Staining Solutions.—A saturated solution of the dye is made with alcohol. This is called the *stock* or *concentrated* solution; 1 part of this solution to about 10 parts of distilled water constitutes the ordinary aqueous solution in use or *weak* solution.

It is readily made by adding to an ounce bottle of distilled water enough of the strong solution until the fluid is still opaque in the body of the bottle, but clear in the neck of the same.

These weak solutions should be *renewed* every three or four weeks, otherwise the precipitates formed will interfere with the staining.

Compound Solutions.—By means of certain chemic agents the intensity of the anilin dyes can be greatly increased.

Intensifiers or Mordants.—Agents that "*bite*" into the specimen, carrying the stain with them, depositing it in the deeper layers, are called mordants or etchers.

Various metallic salts and vegetable acids are used for such purpose.

The mother liquid of the anilin dyes, *anilin-oil*, a member of the aromatic benzol group, has also this property.

Anilin-oil Water.—Anilin-oil is shaken up with water and then filtered; the anilin water so obtained is mixed with the dyes, forming the "anilin-water gentian-violet" or anilin-water fuchsin, etc.

Carbolfuchsin.—Carbolic acid or phenol can be used instead of anilin-oil, and forms one of the main ingredients of Ziehl's or Neelsen's solution, used principally in staining Bacillus tuberculosis. Kühne has a carbol-methylene-blue made similar to the carbolfuchsin.

Alkaline Stains.—Alkalis have the same object as the above agents, namely, to intensify the picture. Potassium hydroxid, ammonium carbonate, and sodium hydroxid are used.

Löffler's alkaline blue and Koch's weak alkaline blue are made with potassium.

Heat.—Warming or boiling the stains during the process of staining increases their intensity.

Decolorizing Agents.—The object after staining is usually overcolored in some part, and then *decolorizing* agents are employed. Water is sufficient in many cases; alcohol and strong mineral acids combined are necessary in some.

Iodin as Used in Gram's Method.—Belonging to this group, but used more in the sense of a protective, is *tincture of iodin.* It picks out certain bacteria, which it coats; prevents *them* from being decolorized, but fades the rest of the picture. Then, by using one of the acid or tissue dyes, a contrast color or double staining is obtained. Many of the more important bacteria are not acted upon by the iodin, and it thus becomes a very useful means of diagnosis.

FORMULAS OF DIFFERENT STAINING SOLUTIONS

I. *Saturated Alcoholic Solution*

Place about 10 grams of the powdered dye in a bottle and add 40 grams of alcohol. Shake well and allow to settle. This can be used as the stock bottle.

II. *Weak Solutions*

Made by adding about 1 part of stock solution (I) to 10 parts of distilled water. This is the ordinary solution in use.

III. *Anilin-oil Water*

Anilin-oil	5	parts
Distilled water	100	" —M.

Shake well and filter. To be made fresh each time.

IV. *Anilin-oil Water Dyes*

Saturated alcoholic solution of the dye	11	parts
Anilin-oil water	100	"
Absolute alcohol	10	" —M.

Can be kept ten days.

V. *Alkaline Methylene-blue*

A. *Löffler's:*

Saturated alcoholic solution methylene-blue	30	parts
Solution potassium hydroxid (1 per cent.)	1	part
Water q. s.	100	parts—M.

B. *Koch's:*

Solution potassium hydroxid (10 per cent.)	2	parts
Saturated alcoholic solution methylene-blue	10	"
Distilled water	2000	" —M.

VI. *Phenol Solutions*

A. *Ziehl-Neelsen:*

Fuchsin (powdered)	1	part
Alcohol	10	parts
5 per cent. solution phenol	100	" —M.

Filter. The older the solution, the better.

B. *Kühne:*

Methylene-blue	1.5 parts
Alcohol	10.0 "
5 per cent. solution phenol	100.0 "

Add the phenol gradually. This solution loses strength with age.

VII. *Gram's Iodin Solution*

Iodin	1 part
Potassium iodid	2 parts
Distilled water	300 " —M.

VIII. *Löffler's Mordant (for Flagella)*

Aqueous solution of tannin (20 per cent.)	10 parts
Aqueous solution ferric sulphate (5 per cent.)	1 part
Aqueous decoction of logwood (1:8)	4 parts.—M.

Keep in well-corked bottle.

IX. *Unna's Borax Methyl-blue*

Borax	1 part
Methyl blue	1 "
Water	100 parts.—M.

X. *Gabbet's Acid Blue (Rapid Stain)*

Methylene-blue	2 parts
20 per cent. sulphuric acid	100 " —M.

XI. *Alkaline Anilin-water Solutions*

Sodium hydroxid (1 per cent.)	1 part
Anilin-oil water	100 parts.—M.

And add—

Fuchsin, or methyl-violet powdered	4 parts

Cork well. Filter before using.

XII. *Roux's Double Stain*

Dahlia or gentian-violet	0.5 part
Methyl-green	1.5 parts
Distilled water	200.0 " —M.

Use as other stains, without *acid*.

XIII. *Neisser's Stain (for Diphtheria)*

Solution I

Methylene-blue	1 part
Alcohol (96 per cent.)	20 parts

Dissolve and add—

Water	950 parts
Glacial acetic acid	50 " —M.

Solution II

Vesuvin	2 parts
Water	1000 " —M.

Stain cover-glasses—(1) Three seconds in solution I; (2) wash in water; (3) three seconds in No. II; (4) wash in water. Body of bacillus, brown; oval granules at each end, blue.

XIV. *Carbolthionin (Nicolle)*

Saturated solution thionin in alcohol (90 per cent.)	10 parts
Aqueous solution phenol (1 per cent.)	100 " —M.

Stain sections one-half to one minute.

XV. *Capsule Stain of Hiss*

Use the following, heated until it steams: Saturated alcoholic solution of gentian-violet or fuchsin	5 parts
Distilled water	95 " —M.

Wash in 20 per cent. solution of cupric sulphate crystals.

XVI. *Capsule Stain of Welch*

(1) Pour glacial acetic acid on film. After a few seconds replace with anilin-water gentian-violet without washing in water. (2) Remove all acid by several additions of stain, and allow it to act for three to four minutes. (3) Wash and examine in salt solution 0.8–2.0 per cent.

XVII. *Romanowsky Stains*

A compound dye originally used for malarial parasites, but now employed in some of its modifications in staining blood-films, bacteria in tissues, and protozoa generally.

The stain is difficult to prepare, and can be purchased of supply houses to better advantage.

The chief modifications are:

Leishman's stain, consisting of a 1 per cent. solution methylene-blue, to which 0.5 per cent. sodium carbonate has been added and allowed to stand for twelve hours in incubator at 65° C., and then ten days at room temperature, and a solution of eosin (1: 1000) in water. Equal parts of these solutions are mixed and allowed to stand for six hours. After it has been washed and dried, the precipitate is dissolved in methyl-alcohol.

Giemsa Stain:

Azur II.—eosin	3 parts	
Azur II	8 "	
Glycerin (pure)	250 "	
Methyl-alcohol	250 "	—M.

Azur is a mixture of methylene-blue and eosin prepared in a special way.

Jenner's Stain.—1.2 per cent. aqueous solution of water-soluble eosin; 1 per cent. aqueous solution methylene-blue (Grubler); equal parts of each. Mix; allow to stand twenty-four hours, wash the precipitate, dry it, dissolve 0.5 gm. in 100 c.c. methyl-alcohol.

J. H. Wright's Stain.—Made in much the same way as Leishman's. The precipitate is not washed, but the satur-

ated methyl-alcohol solution is filtered and further diluted with methyl-alcohol. The stains are used in very dilute form. Where the blood-films or exudates are not first fixed in alcohol, the concentrated stain is allowed to cover the preparation for five to twenty seconds to fix; then water is poured on to dilute and from five to fifteen minutes allowed for staining, the excess removed with water. The stains can be purchased in powder or tablet form, and need only be mixed with methyl-alcohol to be ready for use.

CHAPTER VII

GENERAL METHOD OF STAINING SPECIMENS

Cover-glass Preparations.—The material is evenly spread in as thin a layer as possible upon a cover-glass; then, to spread it still more finely, a second cover-glass is pressed down upon the first and the two slid apart. This also secures two specimens. Before they can be stained, they must be perfectly dry, otherwise deformities will arise in the structure.

Drying the Specimen.—The cover-glass can be set aside to dry, or held in the fingers over the Bunsen burner (the fingers preventing too great a degree of heat). Since most of the specimens contain a certain amount of albuminoid material, it is best in all cases to "fix"—*i. e.*, to coagulate the albumin. This is accomplished by passing the cover-glass (after the specimen is dry) three times through the flame of the burner, about three seconds being consumed in so doing, the glass being held in a small forceps, smeared side up.

The best forceps for grasping cover-glasses is a bent one, bent again upward, near the ends (Fig. 13). It prevents the flame or staining fluid from reaching the fingers.

The object is now ready for staining.

Staining.—A few drops of the staining solution are placed

upon the cover-glass so that the whole specimen is covered, and the stain is left on a few minutes, the time depending upon the variety, the strength of stain, and the object desired. Instead of placing the dye upon the object, the cover-glass can be immersed in a small glass dish containing the solution; or, if heat is desired to intensify or hasten the process, a watch-crystal holding the stain is placed over a Bunsen burner and in it the cover-glass; the cover-glass may be held directly in the flame with the staining fluid upon it, which must be constantly renewed until the process is completed, or the cover-glass can be heated in a test-tube, containing stain solution.

Removing Excess of Stain.—The surplus stain is washed off by dipping the glass in distilled water.

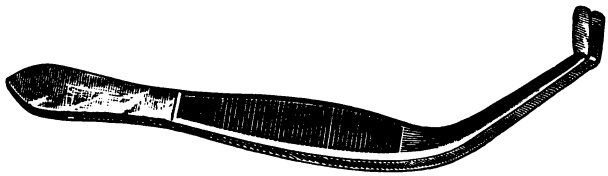


Fig. 13.—Author's bent forceps for holding cover-glass over flame.

The water is removed by drying between filter-paper or simply allowed to run off by standing the cover-glass slantwise against an object. When the specimen is to be examined in water (which is always best with the first preparation of the specimen, as the Canada balsam destroys to some extent the natural appearance of the bacteria), a small drop of sterilized water is placed upon the glass slide, and the cover-glass dropped gently down upon it, so that the cover-glass remains adherent to the slide.

The dry system or the oil immersion can now be used.

When the object has been sufficiently examined, it can be *permanently* mounted by lifting the cover-glass off the slide (this is facilitated by letting a little water flow under it, one

end being slightly elevated). The water that still adheres is dried off in the air or gently over the flame, and when perfectly dry, the cover-glass is placed upon the drop of Canada balsam which has been put upon the glass slide.

In placing the cover-glass in the staining solutions one must be careful to remember which is the spread side, by holding it between one's self and the window and scraping the sides carefully with the sharp point of the forceps, the side having the specimen on it will show the marks of the instrument.

Little glass dishes, about one-half dozen, should be at hand for containing the various stains and decolorants.

Tissue Preparations.—In order to obtain suitable specimens for staining, very thin sections of the tissue must be made.

As with histologic preparations, the tissue must be hardened before it can be cut thin enough. Alcohol is the best agent for this purpose.

Pieces of the tissue one-quarter inch in size are covered with alcohol for twenty-four to forty-eight hours.

When hardened, it must be fixed upon or in some firm object. A paste composed of—

Gelatin	1 part
Glycerin	4 parts
Water	2 "

will make it adhere firmly to a cork in about two hours, or it can be embedded in a small block of paraffin and covered over with melted paraffin. Celloidin may be used as an embedding agent, and formalin is useful to harden tissue quickly.

Cutting.—The microtome should be able to cut sections $\frac{1}{5000}$ inch in thickness; this is the fineness usually required.

The sections are brought into alcohol as soon as cut, unless they have been embedded in paraffin, when they are first washed in chloroform to dissolve out the paraffin.

Staining.—All the various solutions should be in readiness, best placed in the little dishes in the order in which they are to be used, as a short delay in one of the steps may spoil the specimen.

A very useful instrument for transferring the delicate sections from one solution to another is a little metal spatula, the blade being flexible (Fig. 14).

A still better plan, especially when the tissue is "crumbling," is to carry out the whole procedure on the glass slide.

General Principles.—The section is transferred from the alcohol in which it has been kept into water, which removes the excess of alcohol, from here into—

Dish I, containing the *stain*, where it remains five to fifteen minutes. Then—

Dish II, containing 5 *per cent. acetic acid* (1:20), where it

Fig. 14.—Spatula for lifting sections.

remains one-half to one minute. The acid removes the excess of stain.

Dish III, *water*, to rinse off the acid. The section can now be placed under the microscope, covered with cover-glass to see if the intensity of the stain is sufficient or too great. A second section is then taken, avoiding the errors, if any; and having reached this stage, proceeded with as follows:

Dish IV, *alcohol*, two to three seconds, to remove the water in the tissue.

V. A few drops of *oil of cloves*, just long enough to clear the specimen to make it transparent (so that an object placed underneath will shine through).

VI. Remove excess with filter-paper.

VII. Mount in Canada balsam (xylol balsam).

Staining Blood Specimens.—A drop of blood is spread on

a cover-glass and stained with the ordinary dyes; but in order to eliminate the coloring-matter of the red corpuscles and bring the stained bacteria more prominently into view, Gunther recommends that the blood, after drying and fixing, should be rinsed in a dilute solution of acetic acid (1 to 5 per cent.). The hemoglobin is thereby extracted, and the corpuscles appear then only as faint outlines.

Instead of "fixing" by heat, Canon employs alcohol for five minutes, especially in staining for influenza bacilli which have been detected in the blood.

CHAPTER VIII

SPECIAL METHODS OF STAINING AND MODIFICATIONS

Gram's Method of Double Staining (*For Cover-glass Specimens*).—I. A hot solution of anilin-water gentian-violet two to ten minutes.

II. Directly, without washing, into Gram's solution of iodin potassium iodid one to three minutes (the cover-glass looks black).

III. Wash in alcohol 60 per cent. until only a light brown shade remains (as if the glass were smeared with dried blood).

IV. Rinse off alcohol with water.

V. Contrast color with either eosin, picrocarmin, or Bismarck-brown. The bacteria will appear deep blue, all else red or brown on a very faint brown background.

Gram's Method for Tissues (*Modified by Günther*)

I.	Stain in anilin-water gentian-violet	1 minute
II.	Dry between filter-paper.	
III.	Iodin potassium iodid solution	2 minutes
IV.	Alcohol	½ minute
V.	3 per cent. solution hydrochloric acid in alcohol	10 seconds
VI.	Alcohol, oil of cloves, and Canada balsam.	

Behavior of the More Important Bacteria to Gram's Stain.—*Positive* means that the bacteria retain the primary color, or gentian-violet; *negative*, that they do not.

Positive.	*Negative.*
Tubercle bacillus.	Colon bacillus.
Smegma bacillus.	Typhoid bacillus.
Lepra bacillus.	Cholera bacillus.
Anthrax bacillus.	Influenza bacillus.
Tetanus bacillus.	Friedländer's bacillus.
Diphtheria bacillus.	Plague bacillus.
Pneumococcus.	Diplococcus intracellularis.
Streptococcus.	Gonococcus.
Staphylococcus.	Koch-Weeks bacillus.
Cocci of the urethra.	Conjunctivitis bacillus of Morax.

Löffler's Method for Tissues

Alkaline methylene-blue..... 5–30 minutes
1 per cent. acetic acid..... few seconds.
Absolute alcohol, xylol, Canada balsam.
Bacteria dark blue, nuclei blue, cell-bodies light blue.

To Stain Spores.—Since spores have a very firm capsule, which tends to keep out all external agents, a very intensive stain is required to penetrate them, but once this object is attained, it is equally as difficult to decolorize them.

A cover-glass prepared in the usual way, *i. e.*, drying and passing the specimen through the flame three times, is placed in a watch-crystal containing Ziehl's carbolfuchsin solution, and the same placed upon a rack over a Bunsen burner, where it is kept at boiling-point for *one hour*, careful to supply fresh solution at short intervals lest it dry up.

The bacilli are now decolorized in alcohol containing 0.5 per cent. hydrochloric acid. A contrast color, preferably methylene-blue, is added for a few minutes.

The spores will appear as little red beads in the blue-stained bacteria, and loose spores lying about outside the cell-wall.

Spore Stain (Modified).—I. Carbolfuchsin on cover-glass and heated in the flame to boiling-point 20 to 30 times.

II. 25 per cent. sulphuric acid, two seconds; rinsed in water.

III. Methylene-blue contrast.

Alex. Klein recommends the following spore method: mix a little of the culture (potato) with three drops of physiologic salt solution, and heat gently with an equal quantity of carbolfuchsin for a period of six minutes. Spread then on cover-glasses, dry in the air, and fix by passing three times through Bunsen-burner flame. Decolorize in 1 per cent. sulphuric acid for one to two seconds; contrast in weak methylene-blue.

Bowhill's Orcein Stain

Saturated alcoholic solution of orcein . 15 c.c.
20 per cent. aqueous solution tannin . 10 c.c.
Distilled water . . . . . 30 c.c.—M.
Filter.

Use orcein solution in watch-glass, float cover-glass in it, and heat gently, not boil, for ten minutes. Wash in water. Dry and mount in balsam.

Five per cent. chromium trioxid applied for fifteen minutes has been recommended in staining spores. This is followed by the carbolfuchsin stain as above.

Sporogenic bodies stain quite readily, and in order to distinguish them from spores *Ernst* uses alkaline methylene-blue, slightly warmed. Then rinse in water. Contrast with cold Bismarck-brown. The spores are colored bright blue, the spore granules a dirty blue, being mixed with the brown, which colors also the bacteria.

Kühne's Method.—In sections the alcohol used sometimes decolorizes too much. To obviate this *Kühne* mixes the alco-

hol with the stain, so that while the section is being anhydrated, it is constantly supplied with fresh dye.

Weigert uses anilin-oil to dehydrate instead of alcohol, and here, too, it can be used mixed with the dye.

Capsule Stain (*Buerger*).—I. Spread culture by means of a drop of ascitic fluid on cover-glass.

II. Fix in Müller's fluid, which has been saturated with 5 per cent. bichlorid of mercury, and warm for three seconds.

III. Wash quickly in water; rinse in alcohol.

IV. Cover with tincture of iodin for one minute.

V. Wash in alcohol and dry in air.

VI. Stain in anilin-water gentian-violet for two seconds.

VII. Wash in 2 per cent. salt solution.

VIII. Mount in salt solution ringed with vaselin.

Hiss' Method for Capsule.—Smear on cover-glass the organisms mixed with a drop of animal serum (beef-blood serum or ascitic fluid). Dry in air. Fix by heat. Stain for few seconds in Hiss' stain (p. 52). Wash in 20 per cent. copper sulphate solution. Dry and mount. Capsule appears as faint blue halo about dark-purple cell.

Flagella Stain, with Löffler's Mordant.—I. A few drops of the mordant stain (p. 51) are placed upon the spread cover-glass and heated until it steams.

II. Wash with water until the cover-glass looks almost clean, using a small piece of filter-paper to rub off the crusts which have gathered around the edges.

III. Anilin-water fuchsin (neutral) held in flame about one and one-half minutes.

IV. Wash in water.

If the stain is properly made, the bacteria are deeply colored and the flagella seen as little dark lines attached to them.

Unna's Method for Fungi.—Especially useful for epidermic scales. Moisten horny scale or crust with acetic acid; macerate between two glass slides; dry in flame; wash out fat with ether and alcohol (equal parts); stain in *borax methyl-blue*

for ten seconds (over flame); bleach with glycerin and ether (equal parts); rinse in water, alcohol, dry, and mount.

CHAPTER IX

CULTIVATION OF BACTERIA

Artificial Cultivation.—The objects of cultivation are to obtain germs in pure culture, free from all foreign matter, isolated, and so developed as to be readily used either for microscopic examination or animal experimentation.

To develop bacteria properly we supply, as nearly as possible, the conditions which hold for the especial germ in nature. With the aid of solid nutrient media the bacteria can be easily separated, and the methods have been gradually evolved from those originally devised by Pasteur and Koch.

Sterilization of Culture-media, etc.—If we place our nutrient material in vessels that have not been properly disinfected, we will obtain growths of bacteria without having sown any.

If we have thoroughly cleaned our utensils and then not taken care to protect them from further exposure, the germs we have sown will be effaced or contaminated by multitudes of others that are constantly about us. We, therefore, have two necessary precautions to take:

First, thoroughly to clean and sterilize every object that enters into, or in any way comes in contact with, the culture.

Second, to maintain this degree of sterility throughout the whole course of the growth, and prevent, by proper containers, the entrance of foreign germs.

Disinfectants.—Corrosive sublimate (bichlorid of mercury), which is the most effective agent we possess, cannot be generally used because it renders the soil unproductive, and, therefore, must be employed only in washing dishes, to destroy the

old cultures. Even after washing a few drops of the solution may remain and prevent growth, so that one must be careful to have the glassware that comes in contact with the nutrient media free from the sublimate.

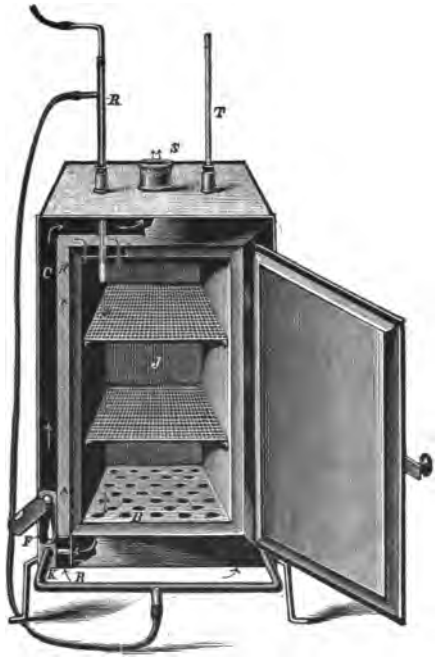


Fig. 15.—Hot-air sterilizer. The gas-jets are inclosed within the space between the outer and middle walls, *C*, and can be seen at *F*. The heat ascends, warming the air between the two inner walls, which ascends between the walls, *K*, *K*, then descends over the contents, *J*, and escapes through perforations in the bottom, *B*, to supply the draft at *F*, and eventually escapes again at *S; R*, gas regulator; *T*, thermometer.

Heat.—Heat is the best agent we possess for general use. Dry heat and moist heat are the two forms employed, but these differ greatly in effectiveness. Thus Koch found that

while moist heat at 100° C. killed the spores of the anthrax bacillus in one hour, it required three hours of dry heat at 140° C. to produce death.

For obtaining *dry heat*—that is, a temperature of 150° C. (about 300° F.)—a sheet-iron oven (Fig. 15) is used which can be heated by a gas-burner. If it have double walls (air circulating between), the desired temperature is much more quickly obtained. A small opening in the top to admit a thermometer is necessary. These chests are usually about 1 foot high, 1¼ feet wide, and ¾ foot deep. In them glassware, cotton, and paper can be sterilized. When the cotton is turned slightly brown, it usually denotes sufficient sterilization. All instruments, where practicable, should be drawn through the flame of an alcohol lamp or Bunsen burner. One hour in the oven at 170° C. usually sterilizes glassware, while the ordinary germs in liquids may be killed by boiling for five minutes if no spores are present. The boiling of any fluid at 100° C. for one and one-half hours nearly always insures sterilization.

Moist Heat.—Steam at 100° C. in circulation has been shown to be a very effective application of heat.

The steam chest devised by Koch consisted of a long double boiler divided by a perforated shelf on which the material could rest while subjected to streaming steam.

Arnold's steam sterilizer will answer every purpose of the Koch steam-chest. It is cheaper, also requiring less fuel to keep it going. The steam does not escape, but is condensed in the outer chamber.

The autoclave (Fig. 16), which produces steam under pressure and allows a temperature of 120° C. to be obtained, is a most effective method of sterilization, but the higher temperatures are not suitable for gelatin or sugar solution. Gelatin loses its power of solidifying if the boiling is prolonged.

Instead of sterilizing for a long time at once, successive sterilization is practised with nutrient media, so that the albumin will not be too strongly coagulated. Fifteen minutes

each day for three days in succession in the Arnold sterilizer, or one exposure in the autoclave, five to fifteen minutes, at 15 pounds pressure; 120° C. is sufficient to sterilize most culture-media.

Fractional Sterilization of Tyndall.—Granted that so many spores originally exist in the object to be sterilized, it

Fig. 16.—Autoclave. Horizontal form.

is subjected to 60° C. for four hours, in which time a part at least of those spores have developed into bacteria, and the bacteria destroyed by the further application of the heat. The next day more bacteria will have formed, and four hours' subjection to 60° C. heat will destroy them, and so, at the end of a week, using four hours' application each day, all the spores originally present will have germinated and the bacteria be destroyed.

As modified, and in use in most laboratories, fifteen minutes, sterilization in steam, at 100° C., in the Arnold sterilizer on three successive days, has been found sufficient, while one sterilization in the autoclave at 120° C. for fifteen minutes will serve in most cases, especially if the medium is for immediate use, and does not contain gelatin or sugar.

Cotton Plugs or Corks.—All the glass vessels (test-tubes, flasks, etc.) must be closed with cotton plugs, cotton-wool,

Fig. 17.—Wire cage.

Fig. 18.—Cotton-plugged test-tubes.

or a good quality of non-absorbent cotton), the cotton being easily sterilized and preventing the entrance of germs from the air.

Tin-foil may be used to cover the cotton, or caps made of india-rubber.

Test-tubes.—New test-tubes are washed with hydrochloric acid and water to neutralize the alkalinity often present in fresh glass, or in chromic acid cleaning mixture one hour. (Potassium dichromate, 6; water, 30; sulphuric acid, 46.) They are then well washed and rubbed with a brush,

placed obliquely to drain, and when dry, corked with cotton plugs. Then put in the hot-air oven (little wire cages, Fig. 17, being used to contain them) for fifteen minutes, after which they are ready to be filled with the nutrient medium. (The cotton should fit firmly in the tube and extend a short space beyond it.)

Test-tubes without flaring edges are more desirable, since the edges can easily be drawn out so as to seal the tube.

Instead of test-tubes, ordinary 3-ounce panel medicine bottles can be used for retaining the nutrient media and cultures.

According to investigations, the glass tubes become sufficiently sterile in the steam-chest without the preliminary sterilization in the dry oven.

Sterilization by Filtration.—*Germ Filters.*—Kaolin or porcelain bougies, such as are used in the Berkefeld, Chamberland, and Pasteur filters, restrain most bacteria, except those now known as ultramicroscopic. In the making of toxins this method is used, heat or disinfectants being undesirable. With the knowledge of smaller forms of life, the filter will need further improvement.

CHAPTER X

PREPARATION OF NUTRIENT CULTURE-MEDIA

Of the many different media recommended and used since bacteriology became a science, we can describe only the more important ones now in use. Each investigator changes them according to his taste.

Potato as Medium.—The knowledge of bacteria and germs or molds settling and growing upon slices of potato

exposed to the air led to the use of solid media for the artificial culture of the same. It was thus learned that each germ tends to form a separate colony and remain isolated, and so pure cultures were first obtained.

Esmarch's Cubes.—The potato is first well cleaned and peeled. It is then cut in cubes ½ inch in size.

These are placed, each in a little glass dish or tray, and then in steam-chest for one-half hour, after which they are ready for inoculation (the dishes first having been sterilized in hot-air oven).

Fig. 19.—Tube for potato culture.

Test-tube Potatoes.—Cones are cut out of the peeled potato and placed in test-tubes, which can then be plugged and easily preserved.

Roux's test-tube (Fig. 19), specially designed for potato cultures, consists of a tube with a small constricted portion at the bottom, in which water may be kept to keep the potato moist.

Manner of Inoculating Potatoes.—With a platinum rod or a spatula (sterilized) the material is spread upon one of the slices, keeping free of the edges. The growth on this first, or original, potato will be quite luxuriant, and the individual colonies often difficult to recognize; therefore dilutions are made.

From the original or first slice a small portion, including some of the meat of the potato, is spread upon the surface of a second slice, which is first dilution. From this likewise a small bit is taken and spread on a third slice, or second dilution, and here usually the colonies will be sparsely enough settled to study them in their individuality.

This is the principle carried on in all the cultivations. It is a physical analysis.

Potato and Bread Mash.—These pastes are used chiefly in the culture of molds and yeasts. Peeled potatoes are mashed with distilled water until thick, and then sterilized

in flasks three-quarters of an hour for three successive days.

Bread Mash.—Bread devoid of crust, dried in an oven, and then pulverized and mixed with water until thick, and sterilized as above.

Solid transparent media are prepared from materials which are transparent and which can readily be converted into liquids. Such are the gelatin and agar culture-media.

Gelatin.—Gelatin is obtained from bones and tendons, and consists chiefly of chondrin and gluten.

Agar-agar.—This agent, which is of vegetable origin, derived from sea-plants gathered on the coasts of India and Japan, has many of the properties of gelatin, retaining its solidity at a much higher temperature; it becomes liquid at 90° C. and congeals again at 45° C. (gelatin will liquefy at 35° C.), whereas 38° C. is the temperature at which most pathogenic germs grow best. Agar cultures can be kept in incubator for days and weeks without liquefying.

Agar is not affected very much by the peptonizing action of the bacteria.

The crude agar should first be rinsed in water, and then in 5 per cent. acetic acid and clear water again, to rid it of impurities. If agar is boiled thoroughly over a hot flame or in an autoclave, it can be filtered much more readily. The main point is to see that all the agar is dissolved.

Glycerin-agar.—The addition of 4 to 6 per cent. of glycerin to nutrient agar greatly enhances its value as a culture-medium.

Gelatin-agar.—A mixture of 5 per cent. gelatin and 0.75 per cent. agar combines in it some of the virtues of both agents.

Blood-serum.—Blood-serum, being rich in albumin, coagulates very easily at 70° C., and if this temperature is not exceeded, a transparent solid substance is obtained upon which the majority of bacteria develop, and some with preference.

PREPARATION OF NUTRIENT CULTURE-MEDIA

(After the recommendations of the American Public Health Association)

Materials.—All water used should be distilled.

Fresh meat.

Dried peptone, Witte brand.

Best French gelatin, as free as possible from impurities.

Best commercial agar in threads.

Sugars, dextrose, lactose, and saccharose, all chemically pure.

Glycerin, double distilled.

Azolitmin in place of litmus.

All other materials as nearly as possible chemically pure.

Sterilization.—Preferably in the autoclave and in small containers, at 120° C., with 15 pounds pressure for fifteen minutes. The sterilizer should be hot before the medium is put in.

Intermittent.—For gelatin or sugar media a high temperature is not suitable. The media are placed in streaming steam for thirty minutes on three successive days.

Reaction.—One-half per cent. solution phenolphthalein (5 grams to 1 liter alcohol) is needed as an indicator. The reaction should be +1 per cent., *i. e.*, 1 per cent. alkaline solution required to make it neutral.

Method of Obtaining Reaction.—To 5 c.c. of medium add 45 c.c. water. Boil one minute. Add 1 c.c. solution phenolphthalein. If the mixture is not tinted pink, the medium is acid or neutral and requires the gradual addition of 1 : 20 normal sodium hydroxid solution until a faint pink color remains. The soda should be added while the mixture is hot or boiling. Calculate from the amount of alkali used for the 5 c.c. how much will be needed for the whole quantity of media and add the same, using normal solution instead of 1 : 20 normal. Example: If 2 c.c. $\frac{N}{20}$ NaOH will neutralize 5 c.c. media, 2 c.c. $\frac{N}{1}$ NaOH will neutralize 100 c.c., or 20 c.c. $\frac{N}{21}$ NaOH will neutralize 1000 c.c. media.

If the medium is very alkaline, hydrochloric acid must be added to reduce to + 1 per cent.

Nitrate Broth.—One gram peptone to one liter water and add 0.2 gm. nitrite free potassium nitrate; place ten c.c. in test-tube, sterilize in autoclave.

Nutrient Broth.—1. Cover 1 pound (500 gm.) chopped meat with 1000 c.c. water and place in refrigerator twelve hours.

2. Strain through Canton-flannel or cheese-cloth and add water to make 1000 c.c.
3. Add 1 per cent. peptone, warming until dissolved.
4. Heat over water-bath thirty minutes.
5. Restore loss of water.
6. Titrate and adjust reaction to +1 per cent. by adding alkali or acid. (See above.)
7. Boil two minutes over free flame.
8. Restore loss of evaporation.
9. Filter through absorbent cotton and Canton-flannel and refilter until clear.
10. Titrate and record final reaction. If it varies 0.2 per cent. from standard, readjust.
11. Tube, using 10 c.c. in each tube.
12. Sterilize.

The nutrient broth as above prepared is used as a basis for most of the other media. It is practically the same as was devised by Löffler in the early days of bacteriology.

Sugar Broths.—Prepared as the standard broth with the addition of 1 per cent. dextrose, lactose, or other sugar just before final sterilization.

Nutrient Gelatin.—Ten per cent. gelatin is added with the peptone to the meat-water infusion. Warm gently at 60° C. until dissolved, then adjust reaction. Heat over steam-bath for forty minutes. Restore loss of evaporation, readjust reaction, and boil five minutes. Make up loss from evaporation and record final reaction. Filter, tube, and sterilize fifteen minutes in autoclave at 120° C. Place at once in ice-water until solid and store in ice-chest.

Nutrient Agar.—Boil 10 to 15 gm. thread agar in 500 c.c.

water for half-hour or digest in autoclave fifteen minutes. Restore loss by evaporation and allow to cool to 60 c.c. To meat-water infusion (500 parts meat to 500 c.c. water) add 2 per cent. peptone, also 500 c.c. agar solution. Titrate after boiling one minute, and adjust reaction to +1. Heat in steam-bath forty minutes, and proceed as with nutrient gelatin, *i. e.*, restore loss, readjust reaction, and filter and refilter until clear. The filtering should be done while the solution is hot. Pour into tubes or plates, sterilize in autoclave, and finally slant the tubes so as to obtain a larger surface. (Most agar tubes are used for stroke cultures.)

Fig. 20.—Flask to receive blood-serum.

The addition of the white of an egg will often clear it up; if this avails not, refiltering several times and attention to the few points mentioned will produce a clear solution.

Lactose Litmus Agar.—One per cent. lactose added to nutrient agar just before sterilization. Reaction neutral. One per cent. azolitmin (Kahlbaum) boiled five minutes and added either to the tube before final sterilization or, if media used in plates, added at the time of plating.

Preparation of Nutrient Blood-serum.—If the slaughter of the animal can be supervised, it were best to have the site of the wound and the knife sterilized, and sterile flasks (Fig. 20) at hand to receive the blood directly as it flows.

The blood is placed on ice forty-eight hours, and the serum is drawn out with sterile pipets into test-tubes, avoiding shaking of the jar. These are placed obliquely in an oven where the temperature can be controlled and maintained. (See Fig. 21.)

Coagulation of Blood-serum.—The tubes of blood-serum

having been placed in the thermostat, are kept at a temperature of 65° to 68° C. until coagulation occurs; then removed and sterilized by fractional sterilization.

Sterilization of Blood-serum.—The tubes are placed three to four days in incubator at 58° C., and those tubes which show any evidences of organic growth are discarded.

If, now, at the end of a week, the serum remains sterile at

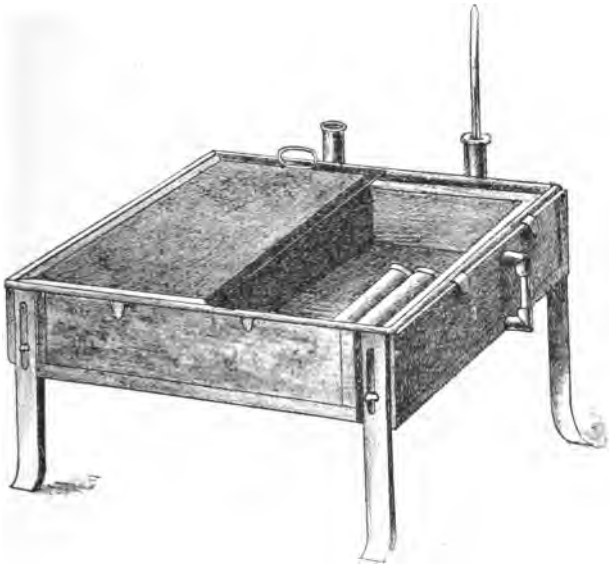


Fig. 21.—Thermostat or inspissator for blood-serum.

the ordinary temperature of the room, it can be used for experimental purposes.

Perfectly prepared blood-serum is transparent, of a gelatin-like consistence, and straw color. It will not liquefy by heat, though bacteria can digest it. Water of condensation always forms, which prevents the drying of the serum.

Short Method.—Blood-serum may be prepared in a shorter

way by coagulating the serum at a temperature short of boiling-point. Sterilization is completed in three days by exposing the tubes to a temperature of about 90° C. each day for five minutes. Tubes so prepared are opaque and white.

Preservation of Blood-serum in Liquid State.—Kirchner advises the use of chloroform. To a quantity of serum in a well-stoppered flask a small amount of chloroform is added—enough to form about a 2 mm. layer on the bottom. If the chloroform is not allowed to evaporate, the serum remains sterile for a long time. When needed for use, test-tubes are filled and placed in a water-bath at 50° C. until all chloroform has been driven off (determined by absence of characteristic odor); the serum is then solidified and sterilized as in the ordinary way, or may be used in a fluid state.

Fig. 22.—Incubator.

Human Blood-serum.—Blood-serum derived from placenta, serum from ascitic fluid and ovarian cysts, is prepared in a similar manner to the above.

Blood coagulum, suggested by the author, is the blood itself (not the serum only) coagulated in test-tubes. It is dark brown in color and allows some colonies of bacteria to be more visible. It requires less time to prepare, and is not so likely to become contaminated as when the serum is used.

Löffler's Blood-serum Mixture.—To 3 parts clear serum add 1 per cent. glucose, beef infusion, and prepare as above; tube.

Hiss' Medium for Plating

Agar	15 gm.
Gelatin	15 "
Meat extract	5 "
Sodium chlorid	5 "
Dextrose	10 "
Distilled water	1000 c.c.

Digest agar in autoclave, then add the other ingredients, except dextrose, which is added to the cleared and filtered product. No neutralization is necessary. Tube in regular way. For tube cultures this medium is modified by using agar 5 gm. and gelatin 80 gm. in place of the quantities given above. A careful titration is made and the reaction adjusted to 1.5 per cent. acid by adding HCl. After filtration, dextrose is added, then tubed and sterilized.

Hesse's Medium for Typhoid

Agar	5 gm.
Peptone	10 gm.
Extract of beef	5 "
Sodium chlorid	8.5 "
Water	1000 c.c.

Digest agar in 500 c.c. water, add the other ingredients dissolved in water. Mix and filter. Adjust reaction to 1 per cent. acid, tube, and sterilize in autoclave.

Bile Salt Agar (MacConkey's)

Sodium taurocholate	0.5 part
Peptone	1.5 parts
Lactose	3.5 "
Agar	1.5 "
Water	q. s. 100.0 "

Agar and peptone dissolved first. Lactose and bile salt added before tubing. Sterilize on three days *intermittently.*

(A) **Conradi-Drigalski Medium**

Fresh meat	1500 gm.
Water	2000 c.c.

Mix and allow to stand twelve hours. Strain, boil one hour, and add—

Peptone	20 gm.
Nutrose	20 "
NaCl	10 "

Boil one hour, filter, then add—

Agar	60 gm.

Boil one hour in autoclave or until agar is dissolved. Render weakly alkaline to litmus, filter, and boil one-half hour.

(B)

Litmus solution (Kahlbaum)	300 c.c.
Lactose	30 gm.

Boil fifteen minutes. Mix with solution A, and make slightly alkaline with soda solution. Then add 4 c.c. 10 per cent. soda carbonate solution (hot sterile) and 20 c.c. of sterile 1 : 1000 crystal violet solution (Höchst B).

Lactose-bile (*Jackson*).—Sterilized undiluted ox-gall, 98 parts; or dry bile, 10 per cent. solution; peptone, 1 part; lactose, 1 part. M. Filled into fermentation tubes, 40 c.c. each, sterilized fractional method.

Blood-agar.—Human or other blood is obtained direct from the body under strict aseptic conditions, and a few drops smeared over the surface of agar in tubes or plates. These are then placed in the incubator for a few days, and the contaminated ones are rejected. This medium is used for influenza bacilli and gonococci.

Elsner's Medium (for Typhoid) (Potassium Iodid—Potato-gelatin).—Five hundred grams of peeled and washed potatoes are mashed and pressed through a fine cloth. The juice is allowed to settle, is filtered, and after one hour's cooking has added to it 10 per cent. gelatin; then

2½ c.c. $\frac{1}{10}$ normal sodium hydroxid solution, and finally 1 per cent. potassium iodid.

Endo Medium (*Fuchsin-Lactose-Agar*).—To 1000 c.c. agar add lactose, 10 grams; fuchsin (saturated alcoholic solution), 2 c.c.; solution sodium sulphite (10 per cent.), 25 c.c.; sterilize in steam, and make acid, 0.1 per cent.

Peptone Water (Modified Dunham) (*Mother Solution*):

Dry peptone (Witte)	100 parts
Sodium chlorid	100 "
Potassium nitrate	1 part
Sodium carbonate	1 "
Distilled water (95)	q. s. ad 1000 parts—M.

When wanted for use, dilute ten times with *water*.

Dunham's rosalic acid solution consists of the following:

Peptone solution (Dunham)	100 c.c.
2 per cent. solution rosalic acid	0.5 gm.
Alcohol (80 per cent.)	100 c.c.—M.

To detect acids and alkalis.

Dieudonné's Medium:

A. Normal solution potassium hydrate, defibrinated ox-blood, equal parts. Mix, sterilize in autoclave.

B. Nutrient agar (neutral). Mix 3 parts A with 7 parts B, and pour into Petri dishes; allow to stand forty-eight hours at room temperature before using.

Milk Culture-medium.—The milk used should be fresh and should be placed on ice for eight to ten hours to allow the cream to rise; the skimmed milk is siphoned off into flasks or tubes and sterilized for three successive days. *Litmus* is often added, or sterile 1 per cent. *azolitmin solution.*

Fresh Egg Cultures (After Hueppe).—The eggs in the shell are carefully cleaned, washed with sublimate, and dried with cotton.

The inoculation occurs through a very fine opening made in the shell with a hot platinum needle; after inoculation, the opening is covered with a piece of sterilized paper and collodion.

Boiled Eggs.—Eggs boiled, shell removed over small portion, and the coagulated albumen stroked with the material.

Guinea-pig Bouillon.—The flesh of guinea-pigs, as well as that of other experiment animals, is used instead of beef in the preparation of bouillon, for the growth of special germs.

The extracts of different organs have been added to the various media for experimentation.

Wertheim's Medium for Gonococcus:

Nutrient agar	2 parts
Human blood-serum or hydrocele fluid	1 part

Melt agar and cool to 45° C.; then add serum. Tube on slant or pour in Petri plate. Glycerin or glucose can be added to *enrich.*

Solution Dried Blood Albumin (King):

Blood albumin (commercial)	15 parts
Glucose bouillon	85 "

Dissolve, tube, inspissate, and sterilize as for blood-serum.

CHAPTER XI

INOCULATION OF CULTURE-MEDIA

Glass Slide Cultures.—Formerly the gelatin was poured on little glass slides, such as are used for microscopic purposes, and after it had become hard, inoculated in separate spots as with potatoes.

Test-tube Cultures.—The gelatin, agar, or blood-serum having solidified in an oblique position is smeared on the surface with the material, and the growth occurs along the smear, or the medium is punctured with a stab of the platinum rod containing the material, and the growth follows the line of thrust. The former is called a *stroke* or *smear culture,* the latter a *stab* or *thrust culture.*

Streaked Surface Plating.—The surface of the medium, hardened in a Petri dish, is scratched by a needle containing the inoculating material, three or more streaks being made without obtaining fresh material, so that the growth along the streak or scratches will represent varying amounts of the substance to be tested. In removing the cotton plugs from the sterile tubes to carry out the inoculation the plugs should remain between the fingers in such a way that the part which comes in contact with the mouth of the tube will not touch anything (Fig. 23).

It is well to pass the mouth of the tube and the cotton plugs through a flame, scorching the latter before reinserting

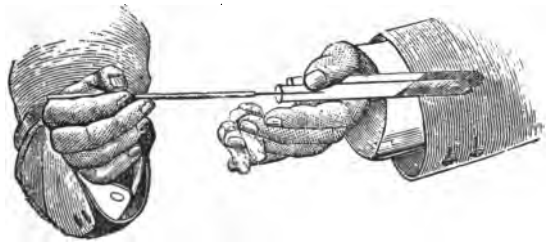


Fig. 23.—Manner of holding plugs.

Sterilizing Needle.—Sterilize needles by passing through the flame before and after each inoculation; also sterilize the glass part, as it is liable to become infected.

After the needle has been withdrawn, the plugs are reinserted and the tubes labeled with the kind and date of culture.

Plate Cultures.—Several tubes of the culture-medium are made liquid by heating in water-bath, and then inoculated with the material as follows. A looped platinum needle is dipped into the material and then shaken in the tube of liquid media (gelatin, agar, etc.).

This first tube is called *original.* From this three drops (taken with the looped platinum rod, Fig. 11, p. 45) are placed in a second tube, the rod being shaken somewhat in the

gelatin or agar; this is labeled *first dilution* (a colored pencil is useful for such markings). From the first dilution three drops are taken into a third tube, which becomes the *second dilution.*

The plugs of cotton must be replaced after each inoculation, and while being held must be carefully protected from contamination.

Glass Plating.—The larger the surface over which the nutrient medium is spread, the more isolated will the colonies be; window glass cut in rectangular plates 6 x 4 inches in size was formerly used, but now Petri dishes consisting of 2 circular glass or porcelain dishes, one fitting over the other as a cover, are universally employed (Fig. 24). They are sterilized, the softened and inoculated agar or gelatin is poured from the test-tube into the dish with as much speed

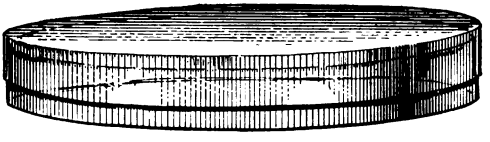


Fig. 24.—Petri dish for making plate cultures.

as possible, and the lid replaced, avoiding contamination from the air and surroundings. They are labeled or marked with pencil, and placed in the incubator or kept at room temperature for further development.

This method is very useful for transportation, and does away with the cooling apparatus and moist chamber formerly employed; the saucers can be viewed under microscope similar to the glass plates, and have entirely superseded them.

Esmarch's Tubes or Rolled Cultures.—This method, especially used in the culture of anaërobic germs, consists in spreading the inoculated gelatin upon the inner walls of the test-tube in which it is contained and allowing it to congeal.

The colonies then develop upon the sides of the tube without the aid of other apparatus. The method is useful whenever a very quick and easy way is required. The rolling of the tube is done under ice-water or running water from the faucet. The tube is held a little slanting, so as to avoid getting too much gelatin around the cotton plug.

The tubes can be placed directly under the microscope for further examination of the colonies.

Animals as Culture-media.—It is almost impossible to separate certain organisms, such as the tubercle bacillus and pneumococcus, from mixed cultures by ordinary plate methods, and the plan of producing the disease in animals by inoculation, and then obtaining the organism in pure culture, has to be employed.

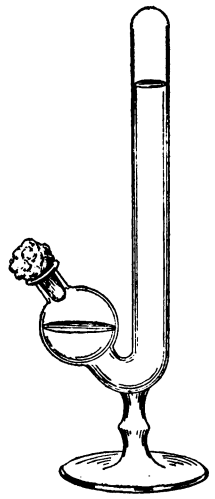


Fig. 25.—Smith's fermentation tube.

Pure Cultures by Boiling.—Spored organisms may be separated from others by boiling the mixture for a few minutes, when all the non-spored forms will perish, and only the spores remain to germinate subsequently.

Fermentation Tube.—For showing the presence of gas or fermentation the Smith tube (Fig. 25) or some of its modifications must be used. The closed end and part of the bulb are filled with the glucose or dextrose bouillon and sterilized at low temperatures for three successive days, then inoculated and placed in the incubator. Gas forms gradually, displacing the fluid in the closed end.

CHAPTER XII

CULTIVATION OF ANAËROBIC BACTERIA

SPECIAL methods are necessary for the culture of the anaerobic variety of bacteria in order to procure a space devoid of oxygen.

Liborius's High Cultures.—The tube is filled about three-quarters full with gelatin, which is then steamed in a water-bath and allowed to cool to 40° C., when it is inoculated

Fig. 26.—Liborius's method.

Fig. 27.—Hesse's method of making anaërobic cultures (McFarland).

by means of a long platinum rod with small loop, the movement being a rotary vertical one, and the rod going to the bottom of the tube.

The gelatin is next quickly solidified under ice; very little air is present. The anaërobic germs will grow from the

bottom upward, and any aërobins present will develop first on top, this method being one of isolation.

From the anaërobic germ grown in the lower part a stab culture is made into another tube containing three-quarters gelatin, the material being obtained by breaking test-tube with the culture. (See Fig. 26.)

Hesse's Method.—A stab-culture having been made with

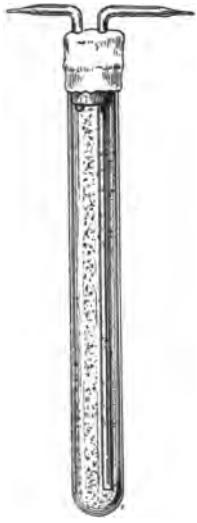


Fig. 28.—Fränkel's method of making anaërobic cultures (McFarland).

Fig. 29.—Buchner's method of making anaërobic cultures (McFarland).

anaërobic germs, gelatin in a semisolid condition is poured into the tube until it is full, thus displacing the air (Fig. 27).

Esmarch's Method.—Having inoculated a tube, the gelatin is rolled out on the walls of the tube, a "roll culture," and the rest of the interior is filled with gelatin, the tube being held in ice-water. The colonies develop upon the sides of the tube and can be examined microscopically.

Gases like Hydrogen to Replace the Oxygen.—Several arrangements for passing a stream of hydrogen through the culture:

Fränkel puts in the test-tube a rubber cork containing two glass tubes, one reaching to the bottom and connected with a hydrogen apparatus, the other very short, both bent at right angles. When the hydrogen has passed through from ten to thirty minutes, the short tube is annealed and then the one in connection with the hydrogen bottle, and the gelatin rolled out upon the walls of the tube (Fig. 28).

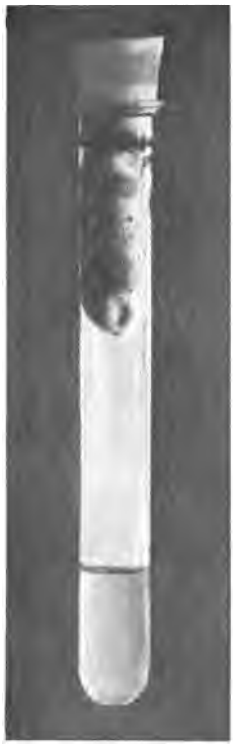


Fig. 30.—Wright's method for the cultivation of anaërobes.

Use of Aërobic Bacteria to Remove the Oxygen.—Roux inoculates an agar tube through a needle-thrust, after which semi-solid gelatin is poured in on top. When the gelatin has solidified, the surface is inoculated with a small quantity of Bacillus subtilis or some other aërobic germ. The subtilis does not allow the oxygen to pass by, appropriating it to itself.

Buchner's Method.—The test-tube containing the culture is placed within a larger tube, the lower part of which contains an alkaline solution of pyrogallic acid. The tube is then closed with a rubber stopper (Fig. 29).

Botkin's Method.—Petri dishes, uncovered, are placed on a rack under a large bell-jar, into which hydrogen gas is conducted. Alkaline pyrogallic acid is placed in the upper

and lower dishes to absorb what oxygen remains. The Novy jar (Fig. 31) is used instead of a bell-jar, and sealed after the oxygen is displaced by hydrogen gas.

Wright's Method.—Applicable to both fluid and solid media. After the test-tube is inoculated the plug, which must be of absorbent cotton, is cut off flush with the extremity of the tube and pushed inward for a distance of 1 cm. It is then impregnated with 1 c.c. of a watery solution of pyrogallic acid and 1 c.c. of 5 per cent. sodium hydroxid

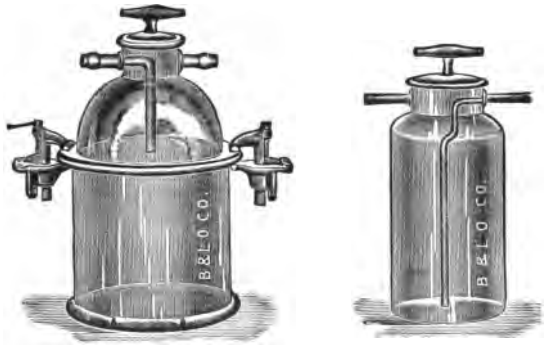


Fig. 31.—Novy's jars for anaërobic cultures.

solution. A tightly fitting rubber stopper is inserted, and the tube is then ready for incubation (Fig. 30).

Park's Method.—An Erlenmeyer flask containing the medium to be used is boiled in a water-bath from ten to fifteen minutes to drive off dissolved oxygen, quickly cooled, and inoculated. Hot melted paraffin is then poured into the flask, which forms a layer over the medium, and on congealing, provides an air-tight seal which does not adhere to the glass so closely as to prevent the escape of any gases formed by the bacterial growth.

Requirements for a Small Laboratory

Incubator, with thermostat and thermometers.
Hot-air oven.

Arnold steam sterilizer.
Autoclave.
Bunsen burners.
Erlenmeyer or liter glass flasks, ½ dozen.
Test-tubes, 100.
One 1000 c.c. measuring glass.
One 100 c.c. measuring glass.
One 5 c.c. pipet.
One 1 c.c. pipet.
One accurate buret.
One-half dozen 20 c.c. porcelain capsules.
Glass stirring rods.
Normal soda solution.
Hydrochloric acid.
Lactose, dextrose, glucose, and phenolphthalein.
A selection of dry stains, especially fuchsin, methylene-blue, and eosin.
Gram's solution.
Phenol.
Alcohol, methyl alcohol.
Cover-glasses, slides.
Canada balsam, cedar-oil, xylol.
A small microtome and embedding material.
Cotton-wool for plugs.
Twenty-five or more Petri dishes.
Four platinum needles in glass handles.
One-half dozen fermentation tubes.
One-half dozen tubes for potato culture.
One Novy jar.
One animal holder.
Three wire boxes for holding tubes.
Test-tube rack.

The materials must include what is needed for making culture-media: agar, gelatin, peptone, beef-extract, chemically pure salt.

And to this there will be added from time to time such other apparatus and material as occasion demands.

CHAPTER XIII

THE GROWTH AND APPEARANCES OF COLONIES

Macroscopic.—Depending greatly upon the temperature, which should be about 65° F. (20° C.) for gelatin, and 40° C. for agar, the colonies ordinarily develop so as to be visible to the naked eye in two to four days. Some require ten to fourteen days, and others grow rapidly, covering the third dilution in thirty-six hours. The plate should be looked at each day.

The colonies present various appearances from that of a

Fig. 32.—Staphylococcus pyogenes aureus: colony two days old, seen upon an agar-agar plate (×40) (Heim).

small dot, like a fly-speck, to that resembling a small leaf. Some are elevated, some depressed, and some, like cholera, cup-shaped—umbilicated.

Then they are variously pigmented. Some liquefy gelatin speedily, others not at all. The appearances of a few are so characteristic as to be recognized at a glance. Some produce gas-bubbles.

Microscopic.—Use a low-power lens, with the Abbé nearly shut out—that is the narrowest blender. The stage of the microscope should be of such size as to carry a Petri saucer easily upon it.

The second dilution or third plate is usually made use of—that one containing the colonies sufficiently isolated.

These isolated ones should be sought for, and their appearance well noticed.

There may be two or three forms from the same germ, the difference due to the greater or less amount of oxygen that

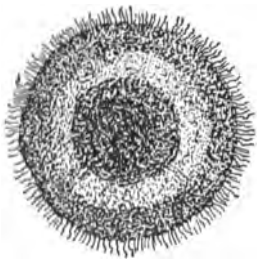


Fig. 33.—Microscopic appearances of colonies.

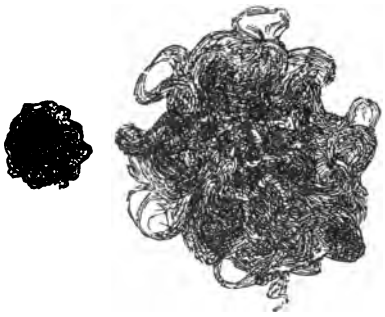


Fig. 34.—Klatsch preparations.

they have received, or the greater or less amount of space that they have had to develop in.

The microscopic picture varies greatly; now it is like the gnarled roots of a tree, and now like bits of frosted glass; some bacteria have quite characteristic colonies (Fig. 32).

Impression or "Klatsch" Preparations.—In order more thoroughly to study a certain colony and to make a permanent specimen of the same, we press a clean cover-glass upon the particular colony, and it adheres to the glass. It can then be stained or examined. The Germans give the name of "Klatsch" to such preparations.

Fishing.—To obtain and examine the individual members

of a particular colony the process of fishing, as it is called, is resorted to.

The colony having been placed under the field of the micro-

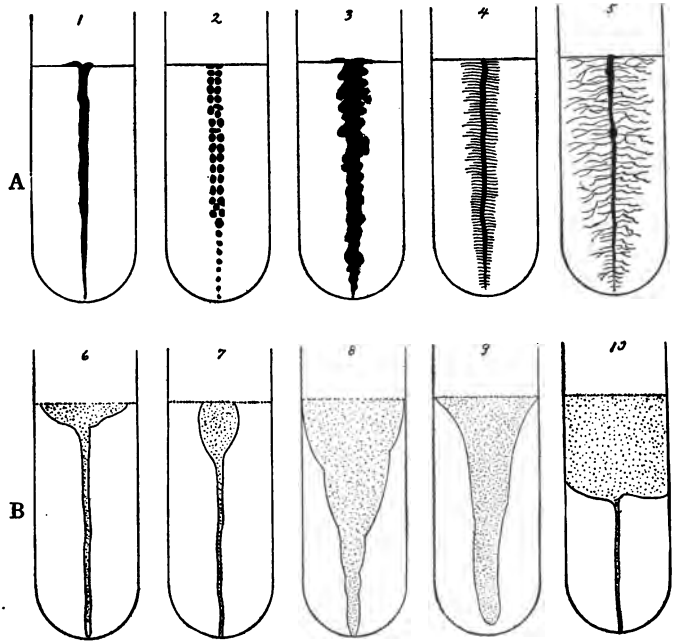


Fig. 35.—Types of growth in stab-cultures: A, Non-liquefying: 1, Filiform (Bacillus coli); 2, beaded (Streptococcus pyogenes); 3, echinate (Bacterium acidi lactici); 4, villous (Bacterium murisepticum); 5, arborescent (Bacillus mycoides). B, Liquefying: 6, Crateriform (Bacillus vulgare, twenty-four hours); 7, napiform (Bacillus subtilis, forty-eight hours); 8, infundibuliform (Bacillus prodigiosus); 9, saccate (Microsporon Finkleri); 10, stratiform (Psorospermum fluorescens) (Frost).

scope, a long platinum needle, the point slightly bent, is passed between the lens and the plate so as to be visible through the microscope, then turned downward until the colony is seen to be disturbed, and the needle is dipped into

the colony. This procedure must be carefully done, lest a different colony be disturbed than the one looked at, and an unknown or unwanted germ obtained.

After the needle has entered the particular colony, it is withdrawn, and the material thus obtained is further examined by staining and animal experimentation. The bacteria

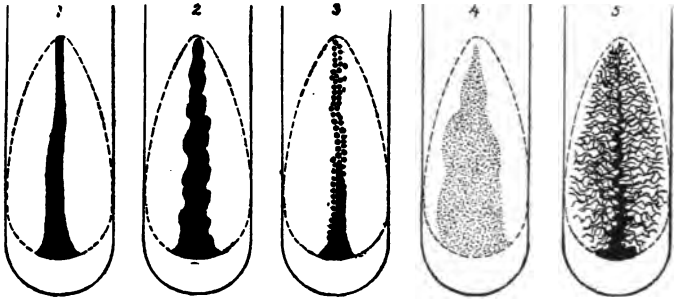


Fig. 36.—Types of stroke cultures: 1, Filiform (Bacillus coli); 2, echinulate (Bacterium acidi lactici); 3, beaded (Streptococcus pyogenes); 4, effuse (Bacillus vulgaris); 5, arborescent (Bacillus mycoides) (Frost).

are further cultivated by inoculating fresh gelatin or agar, making *stab-* and *stroke* cultures.

It is necessary to transfer the bacteria to fresh media about every six weeks, as the products of growth and decay given off by the organisms destroy them. Stroke and stab test-tube cultures are more characteristic than plate cultures, as the types in Figs. 35 and 36 show.

CHAPTER XIV

ANIMAL INOCULATION

USED: (1) For obtaining pure cultures; (2) to determine virulence; (3) to regain virulence of an organism that has become exhausted in artificial media; (4) to furnish a suitable culture-medium for bacteria that have so far failed to grow on other media.

The smaller rodents and birds are the ones usually employed for inoculation, as rabbits, guinea-pigs, rats, mice, pigeons, and chickens. These are preferred, because easily affected by the various bacteria, readily obtained, and not expensive. Monkeys have been used in recent years in connection with syphilis and meningitis.

The white mouse is very prolific and easily kept, and is therefore a favorite animal for experiment. It lives well upon a little moistened bread. A small box, perforated with holes, is filled partly with sawdust, and in this ten to twelve mice can be kept. When the female becomes pregnant, she should be removed to a glass jar until the young have opened their eyes, because the males, which have not been raised together, are apt to attack each other.

Guinea-pigs.—When guinea-pigs have plenty of light and air, they multiply rapidly. Therefore it is best to have them in some large stall or inclosure. They can be fed upon all sorts of vegetables and grasses, and require but little attention.

Methods of Inoculation.—*I. Inhalation.*—Imitating the natural infection, either by loading an atmosphere with the germs in question or by administering them with a spray.

II. Through skin or mucous membrane.

III. With the food.

Method of Cutaneous Inoculation.—The ear of a mouse is best suited for this procedure: A small abrasion is made with the point of a lancet or needle, which has been dipped in the virus or material to be inoculated. The animal is then sepa-

rated from the rest and placed in a glass jar, which is partly filled with sawdust and covered with a piece of wire gauze.

Subcutaneous.—The root of the tail of a mouse is used for this purpose. The hair around the root of the tail is clipped off, and with a pair of scissors a very small pocket is made in the subcutaneous connective tissue, not wounding the animal any more than is absolutely necessary, avoiding much blood. The inoculating material is placed upon a platinum needle and introduced into the pocket; solid bodies, with a forceps.

To hold the mouse still while the operation is going on a little cone made of metal is used. The mouse just fits in here. There is a slit along the top in which the tail can be fastened, and thus the animal is secure and immobile.

Variously designed animal-holders are on the market and used in laboratories.

Intravenous Injections.—Rabbits are very easily injected through the veins. Mice are too small.

The ear of the rabbit is usually taken. It is first washed with 1 : 2000 bichlorid, which not only disinfects, but also makes the vessels appear more distinct. The base of the ear is compressed to swell the veins. Then a hypodermic syringe, which can be easily sterilized, is filled with the desired amount of virus, which is slowly injected into any one of the more prominent veins present (Fig. 37).

Intraperitoneal Injection.—This is used with guinea-pigs chiefly. The abdominal wall is pinched up through its entire thickness, and the needle of the syringe thrust directly through, so that it appears on the other side, then the fold let go, the needle withdrawn just far enough so as to be within the cavity.

Inoculation in the Eye.—The anterior chamber and the cornea are the two places used. The rabbit is fixed upon a board, the eyelids held apart and head held still by an assistant. A few drops of cocain having first been introduced in the eye, a small cut is made in the cornea. The material is passed through the opening with a small forceps, and with a few strokes of a spoon it is pushed in the anterior chamber.

For the cornea a few scratches made in the corneal tissue will suffice; the material is then gently rubbed in.

Inoculation of the Cerebral Membranes.—The skin and aponeurosis cut through where the skull is the thinnest. Then the bone carefully trephined, and the dura exposed. In *rabies* inoculation, the syringe containing the hydrophobic virus pierces the dura and arachnoid, and the virus is discharged beneath the latter.

Fig. 37.—Method of making an intravenous injection into a rabbit. Observe that the needle enters the posterior vein from the hairy surface.

Intratracheal.—The bacteria can be introduced directly into the trachea, thus coming in contact with the lungs.

Intraduodenal.—Cholera germs are injected into the intestines after they have been exposed by carefully opening the abdomen. This is done in order to avoid the action of the gastric juice.

Celloidin sacs of small size are sometimes used to intro-

duce living cultures of bacteria into the bodies of animals without their coming into direct contact with the tissues.

Obtaining Material from Infected Animals.—The animal should be skinned, or the hairs plucked out, before it is washed—at least the portion where the incision is to be made. Then the entire body is washed in sublimate. Two sets of instruments are required—one for coarser and one for finer work: the one sterilized in the flame; the other, to prevent being damaged, heated in a hot-air oven.

The animal, the mouse, for example, is stretched upon a board, a nail or pin through each leg, and the head fixed with a pin through the nose. The skin is dissected away from the belly without exposing the intestines. Then the ribs, being laid bare, the sternum is lifted up, and the pericardium exposed. A platinum needle dipped into the heart after the pericardium has been slit will give sufficient material for starting a culture. If the other organs are to be examined, further dissection is made. If the intestines are first to be looked at, they should be laid bare first.

In this manner material is obtained and the results of inoculation noted.

Frequent sterilization of the instruments is desirable.

Koch's Rules in Regard to Bacterial Cause of Disease.—Before a microbe can be said to be the cause of a disease, it must—

First, be found in the tissue or secretions of the animal suffering from, or dead with, the disease.

Second, it must be cultivated outside of the body on artificial media.

Third, a culture so obtained must produce the disease in question when it is introduced into the body of a healthy animal.

Fourth, the same germ must then again be found in the animal so inoculated.

CHAPTER XV

BACTERINS (VACCINES)

Bacterins are sterilized suspensions of bacteria in normal saline solution. The term *vaccines* or *bacterial vaccines* is frequently but erroneously used in place of bacterins, as the word vaccine relates to a cow or calf. Bacterins are used in the treatment of localized infections, and especially those of a chronic nature, and have been employed extensively to establish immunity against infection. The best example of this is the immunization of armies and inmates of institutions against typhoid fever.

Preparation.—The organism is grown on the surface of the most appropriate medium, usually agar-agar, until an abundant growth is present. This ordinarily requires twenty-four hours. The growth is then washed from the medium with sterile normal saline solution, and collected in a small sterilized flask or bottle containing glass beads and shaken to break up clumps. A sterilized glass bulb, drawn to a point (a test-tube drawn out answers as well), is filled with the resulting emulsion, the end sealed in a flame, and the bulb immersed in a water-bath at 60° C. for one hour. The neck of the bulb is then broken, and a few drops of the emulsion sown on culture-media to determine the presence or absence of living organisms.

Standardization.—The number of bacteria in a cubic centimeter of the mixture is determined as follows: a portion of the emulsion is reserved unheated, and at once mixed with an equal volume of blood by aspirating into a capillary tube any quantity, usually a column 2.5 cm. long, of the emulsion, followed by an equal volume of blood. The blood and emulsion are then mixed on a glass slide and thin smears are made. After air drying, the films are fixed with saturated solution of bichlorid of mercury and stained with carbolthionin.

Counting.—Two crossed hairs are placed on the diaphragm in the eye-piece of the microscope and the slide examined under the oil-immersion lens. The number of corpuscles and bacteria in a number of fields are counted until at least 200 red corpuscles have been enumerated. As the number of corpuscles per cubic centimeter is 5,000,000,000 by simple proportion, the number of bacteria per cubic centimeter can be determined. For example, 200 red corpuscles and 150 bacteria are counted in the same fields. Then—

200 corpuscles : 150 bacteria : : 5,000,000,000 : x x=3,750,000,0000
(Number of corpuscles is to number of bacteria as the total number of corpuscles in a cubic centimeter is to the quantity to be determined.)

Any number of bacteria per cubic centimeter can then be obtained by simple dilution with sterile normal saline solution. When the final dilution is made, 0.2 per cent. of trikresol is added as a preservative.

PART II

SPECIAL BACTERIOLOGY

CHAPTER XVI

SOME COMMON BACTERIA SLIGHTLY PATHOGENIC

Bacterium Prodigiosum (Ehrenberg).—This bacillus, formerly called micrococcus, is very common, and was one of the first noticed, because of the brilliant red pigment it forms on cooked vegetables and starchy substances. "The bleeding host" miracles are said to have been due to it.

Morphology.—Short rods, often in filaments, resembling cocci, ends slightly pointed, 1 μ in size; spores absent.

Facultative anaërobic, that is, it can grow without air; but the pigment requires oxygen for its development.

Flagella and motion present in young bouillon cultures. Absent in older and those grown on potato.

Stain easily with ordinary watery stains, but not with *Gram.*

Cultural Features.—Agar stroke: Growth limited to stroke; filiform, varying from a light pink to dark purple in color, due to pigment (prodigiosin) formed by the growing colonies. Odor of trimethylamin present. Media colored brown underneath growth.

On *potato*, growth of pigment appears best. At first rose red, then in a few days dark purple, with a glistening, green-gold luster, resembling the dry fuchsin dye. Odor more pronounced.

Gelatin Stab.—In six hours liquefaction begins on surface, and spreading downward; funnel shape; the liquid portion

containing small flakes of red pigment which settle at the bottom. *Milk* coagulated in twenty-four hours.

Agar Colonies.—Small red points in thirty-six hours, irregular in outline. *Granular* in structure.

Gelatin Colonies.—On the surface, round, granular, smooth edges which soon liquefy and have depression in center. The edges then become irregular.

Biologic Features.—The characteristic *red pigment* is insoluble in water, slightly soluble in alcohol and ether; alkalies turn it orange, *acids, violet red.* Light fades it. Gases of methylamin and ammonia are produced. Gas and acid produced in sugar solutions. *Indol feeble.*

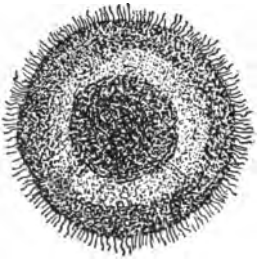


Fig. 38.—Colony of Bacillus mesentericus vulgatus.

Temperature, 22°–25° C.; higher temperatures interfere with pigment.

Pathogenic for small animals. When injected intraperitoneally, 1–2 c.c. has proved fatal; causes intoxication. Proteids of the cultures *poisonous.*

Cancer Remedy. — Used in Coley's treatment mixed with cultures of streptococci.

Bacillus Mesentericus Vulgatus (*Bacillus Vulgatus; Potato Bacillus of Flügge*) (Fig. 38).

Origin.—Surface of the soil, on potatoes, and in milk.

Form.—Small thick rods with rounded ends, often in pairs. Very motile; produces abundant spores.

Cultures.—Rapid growth; stain with *Gram.*

Agar Colonies.—Round, with transparent center at first, then becoming opaque. The border is ciliated; little projections evenly arranged.

Potato.—A white covering at first, which then changes to a rough brown skin; the skin can be detached in long threads.

Temperature.—Spores at ordinary temperature.

Spores.—Are very resistant; are colored in the manner described in first part of the book for spores in general.

Bacillus Megaterium (de Bary) (Fig. 39).—*Origin.*—Found on rotten cabbage and garden-soil.

Form.—Large rods, four times as long as they are broad, 2.5 μ. Thick, rounded ends. Chains with ten or more members often formed; granular cell contents.

Abundant spore formation; very slow movement.

Growth.—Strongly aërobic; grows quickly and best at a temperature of 20° C.

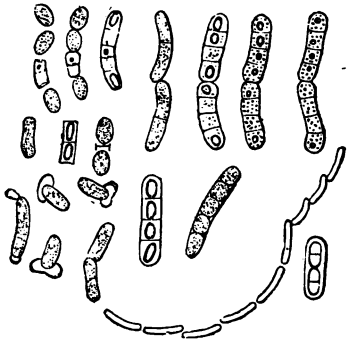


Fig. 39.—Bacillus megaterium, with spores.

Plate Colonies.—Small, round, yellow points in the depth of the gelatin. Under microscope, irregular masses like *B. subtilis.*

Stab-culture.—Funnel-shaped from above downward.

Potato.—Thick growth with abundance of spores like *B. subtilis.*

Bacillus Ramosus.—*Synonyms.*—*Bacillus mycoides* (Flügge); *Wurzel or root bacillus.*

Origin.—In the upper layers of garden or farm grounds and in water.

Form.—Short rods, with rounded ends, about three times as long as they are thick; often in long threads and chains.

Immotile.

Stain.—Gram.

Agar Stroke.—Gray soft mass, gnarled and twisted; feathery extensions spreading over entire surface.

Gelatin Stab.—Arborescent and plumose-parallel projections on either side of the stab; a thick skin on surface with slow liquefaction (Fig. 40).

Colonies.—Twisted threads, like a bundle of hair; opaque center; the threads or branches divide endlessly, forming coils.

Fig. 40.—Bacillus mycoides (Frost).

Growth.—At ordinary temperature, with plentiful supply of air.

Staining.—Spores stain readily with the ordinary spore stain.

Bacterium Zopfii (Kurth) (1883).—*Origin.*—Intestines of a fowl.

Form.—Short thick rods forming long threads coiled up, which finally break up into spores, which were once thought to be micrococci.

Properties.—Very motile; does not dissolve or liquefy gelatin. Produces putrefaction in albuminous media, with gas formation.

Growth.—In thirty hours abundant growth; *aërobic;* grows best at 20° C.

Agar Plates.—Small white points which form the center of a very fine netting. With high power this netting is found composed of bacilli in coils, like braids of hair.

Excellent impress or "Klatsch" preparations are obtained from these colonies.

Staining.—Ordinary dyes and Gram.

Bacillus Subtilis (Hay Bacillus) (Ehrenberg).—*Origin.*—Hay infusions; found also in air, water, soil, feces, and putrefying liquids. Very common, often contaminates cultures.

Form.—Short, thick rods, three times as long as broad; slight roundness of ends; seldom found singly; usually in

long threads. *Flagella* are found on the ends. *Spores* of oval shape, strongly shining, very resistant.

Very motile; *Gram stain.*

Growth.—Rapid; strongly aërobic.

Plate.—Round, gray colonies with depressed white center. Under microscope the center yellow; the periphery like a wreath, with tiny little rays projecting; very characteristic.

Agar Stroke.—Soft, round, smooth edges; gray.

Gelatin Stab.—Gray on surface, sinks in thirty-six hours, shallow crater, in which small white particles are floating; as gelatin softens a skin forms on surface.

Potato.—Thick, dirty-white growth, spreading over surface; *dull, raised* edges, wavy.

Properties like *B. vulgatus.*

Pathogenic.—Has been found present in eyeball suppurations, especially *panophthalmitis.* Injected in guinea-pigs it causes toxemia and death. Has been found in acute conjunctivitis, and may at times produce it.

Staining.—Rods, ordinary stain; *spores,* spore stain.

It is easily obtained by covering finely cut hay with distilled water, and boiling a quarter of an hour. Set aside forty-eight hours. A thick scum will show itself on the surface, composed of the subtilis bacilli, whose spores alone have survived the heat.

Was formerly considered a non-virulent form of *B. anthrax.*

Boas-Oppler Bacillus.—Also known as the Bacillus geniculatus. Owing to the faculty possessed by this organism of growing in the presence of amounts of lactic acid sufficient to check the development of all other lactic-acid formers, it usually predominates in stomach-contents containing large amounts of this substance. The parent type is composed of short rods, but in the presence of considerable amounts of lactic acid these change to a longer form, which occurs singly or in long chains. It is stained brown by Gram's iodin solution. The bacillus affords confirmatory evidence of the presence of a new-growth, like cancer of the stomach, though it may occur in benign conditions.

Bacillus Violaceus (Schrater).—*Origin.*—Water.

Synonym.—*B. ianthinum* (Zopf).

Form.—A slender rod with rounded ends, three times as long as it is broad, often in threads.

Spores.

Motile, flagella.

Stain.—With Gram and ordinary dyes.

Cultures.—*Agar stroke*, moist, glistening, raised, at first yellow, then violet, inky colored.

On Potato.—Violet black, moist, abundant growth.

Gelatin Stab.—Rapidly liquefying funnel-shaped masses of pigment along the stab.

Colonies.—Hairy outer zone with liquid center, and small masses of opaque blue pigment floating about.

Biology.—Acid formed in sugar bouillon. No gas. A moderate amount of H_2S and indol. Pigment formed is insoluble in water, slightly soluble in alcohol.

Facultative anaërobe.

Temperature.—22°–25° C.

Microörganisms Found in Urine.—When freshly passed, urine of a normal state contains no bacteria. By contact with the air and the urinary passages exposed to air, a great number of yeasts, molds and bacteria soon accumulate in the fluid. Bacteria also enter urine through the blood and during its secretion.

A number of bacteria have the property of converting urea into carbonate of ammonia.

The urine should be centrifuged and the deposit then examined. The drying and fixing must proceed very slowly, since otherwise crystals of salts will be precipitated and mar the specimen.

B. coli are frequently present, especially in acid urine.

Typhoid bacilli in 25 per cent. of patients affected with typhoid fever.

Micrococcus Ureæ (Pasteur and Van Tiegham).—*Origin.*—Decomposed urine and in the air.

Form.—Cocci, diplococci, and streptococci.

Properties.—Decomposes urea into ammonium carbonate; does not liquefy gelatin.

Growth.—Grows rapidly, needing oxygen; can remain stationary below 0° C., growing again when a higher temperature is reached.

Colonies on Plate.—On the surface like a drop of wax.

Stab-cultures.—Looks like a very delicate thread along the needle-thrust.

Other bacteria are found in urine in various pathologic processes, such as tubercle bacilli, typhoid bacilli, gonococci, and other pyogenic organisms.

Spirilla.—A number of non-pathogenic spirilla have been described.

Spirillum Rubrum (Esmarch).—*Origin.*—Body of a mouse dead with septicemia.

Form.—Spirals of variable length, long joints, flagella on each end; no spores.

Properties.—Does not liquefy gelatin; very motile; produces a wine-red pigment, which develops only in absence of oxygen.

Growth.—Can grow with oxygen, but is then colorless; grows very slowly; ten to twelve days before any sign; grows best at 37° C.

Gelatin Roll-cultures.—Small, round; first gray, then wine-red colonies.

Stab-cultures.—A red-colored growth along the whole line; it is deepest below, getting paler as it approaches the surface.

Sarcina.—*Cocci* in cubes or packets of colonies. A great number have been isolated, many producing very beautiful pigments. The majority of them found in the air.

Sarcina Lutea (Schröter).—*Origin.*—Air.

Form.—Very large cocci in pairs; tetrads and groups of tetrads.

Properties.—Liquefies gelatin slowly; produces sulphur-yellow pigment.

Growth.—Slowly, at various temperatures; strongly aërobic.

Plates.—Small, round, yellow colonies.

Stab-cultures.—Grows more rapidly, the growth being nearly all on the surface, a few separated colonies following the needle-thrust for a short distance. *Agar*, a very beautiful yellow, along the stroked surface.

Sarcina Aurantica.—*Flava, rosea,* and *alba* are some of the other varieties. Many are obtained from beer.

Sarcina Ventriculi (Goodsir) (Fig. 41).—*Origin.*—Stomach of man and animals.

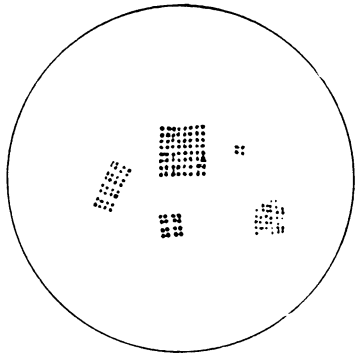


Fig. 41.—Sarcina ventriculi from stomach-contents (× 530) (Van Valzah and Nisbet).

Form.—Colorless oval cocci, in groups of eight and packets of eight.

Properties.—Does not liquefy gelatin; shows tne reaction of *cellulose* to iodin.

Growth.—Rapid. At end of thirty-six hours, round, yellow colonies, from which colorless cocci and cubes are obtained.

Habitat.—They are found in many diseases of the stomach, especially when dilatation exists. Also normally; increased when fermentation occurs.

CHAPTER XVII

BACILLUS OF ANTHRAX

Bacillus Anthracis (Rayer and Davaine).—Rayer and Davaine, in 1850, first described this bacillus; but *Pasteur*, and later *Koch*, gave it the importance it now has.

Synonyms.—Bactericie du charbon (Fr.); *Milzbrand bacillus* (German); *bacillus of splenic fever or malignant pustule.*

Origin.—In blood of anthrax-suffering animals.

Fig. 42.—Bacillus anthracis, stained to show the spores (× 1000) (Fränkel and Pfeiffer).

Form.—Rods of variable length, largest of pathogenic organisms 4 μ to 10 μ in length, nearly the size of a human blood-corpuscle; broad, cup-shaped ends; in bouillon cultures long threads are formed, with *large oval spores* (Figs. 42, 43).

Spores.—Single, large, very resistant. Dry heat, 140° C., in three hours; steam in five minutes; necessary to kill. Do

not occur in the circulating blood, but develop after death or in artificial media at 30° C.

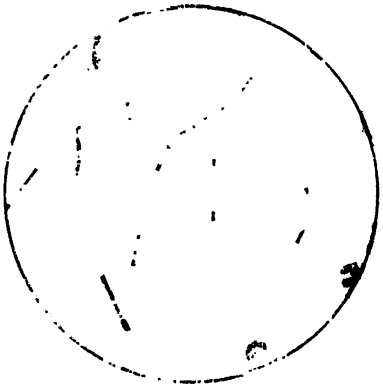


Fig. 43.—Anthrax bacilli in human blood (fuchsin staining) (Zeiss one-twelfth oil-immersion; No. 4 ocular) (taken from Vierordt).

Fig. 44.—Bacillus anthracis, impression preparation, edge of colony; Zettnow prep. (Kolle and Wassermann).

Liquefies gelatin; immotile.

Growth.—Grows rapidly, between 12° C. and 45° C., and

requires plenty of oxygen, but may be classed as a *facultative anaërobe;* grows well in all media.

Colonies develop in two days; white shiny spots, which appear under microscope as slightly yellowish, granular, twisted balls, like a ball of yarn; each separate string or hair, if looked at under high power, being composed of bacteria in threads. (See Fig. 44.)

Fig. 45.

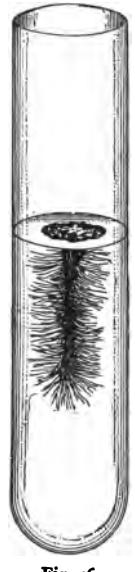


Fig. 46.

Figs. 45, 46.—Stab-cultures of anthrax in gelatin.

Agar Stroke.—Grayish-white, slightly wrinkled layer with irregular edges.

Gelatin Stab-cultures.—A white growth with thorn-like processes along the needle-track (like an "inverted fir tree"). Later on, gelatin liquefied, and flaky masses at the bottom. (See Figs. 45, 46.)

Potato.—A dry, creamy layer, and when placed in incubator, rich in spores.

Staining.—Readily take the anilin dyes with the ordinary methods. To bring out the cup-shaped concave extremities, a very weak watery solution of methylene-blue is best. *Gram positive.*

Spores are stained by the usual method. When several bacilli are joined together, the place of their joining looks like a spore, because of the hollowed ends. Double staining will differentiate the spores. (See Fig. 42.)

Sections of tissue are stained according to the ordinary methods, taking *Gram's* method very nicely.

Pathogenesis.—When mice are inoculated with anthrax material through a wound in the skin, they die in twenty-four hours from an active septicemia, the point of inoculation remaining unchanged.

On autopsy will be found:

Peritoneum.—Covered with a gelatinous exudate.

Spleen.—Very much swollen, dark red, and friable.

Liver.—Parenchymatous degeneration.

Blood.—Dark red. The bacilli are found wherever the capillaries are spread out, in the spleen, liver, intestinal villi, and glomeruli of kidney, and in the blood itself. Only when the capillaries burst are they found in the tubules of the kidney.

Mode of Entrance.—The bacilli can be *inhaled*, and then a pneumonia is caused, the pulmonary cells containing the bacilli; when the spores are inhaled, a general infection occurs.

Feeding.—The cattle graze upon the meadows, where the blood of anthrax animals has flowed and become dried; the resistant spores contaminate the grass and so enter the alimentary tract; here they then cause the intestinal form of the disease, ulcerating through the villi. Cattle are also infected by wading in streams which tannery washings have contaminated.

Local Infection.—In man usually only a local action occurs; by reason of his occupation—woolsorter, cattle-driver, tanner, etc.—he handles the hides or wool of animals that

have been infected, and through a scratch or slight wound he becomes infected, and local gangrene and necrosis set in, but death follows in the severer forms from a general pyemia; there is severe edema of the tissues in and about the wound, and pulmonary edema. Wounds about the face and neck are more fatal.

Pneumonia by inhalation and *intestinal infection* also occur in man.

Woolsorter's disease is the pulmonary form caused by inhalation of spores from infected wool.

Susceptibility of Animals.—Dogs, birds, and cold-blooded animals affected the least; white mice, sheep, and guinea-pigs quickly and surely.

Products of Anthrax Bacilli.—A basic ptomain has not been found, but a toxalbumin or proteid, called *anthraxin*, has been obtained. A certain amount of *acid* is produced by the virulent form, *alkali* by the weak.

Attenuation and Immunity.—Cultures left several days in an incubator at a temperature between 40° and 42° C. soon become innocuous, and when injected into animals protect them against the virulent form.

The lymph obtained from lymph-sac of a frog destroys the virulence of anthrax bacilli and spores temporarily.

Hankin obtained an alexin from the blood and spleen of rats, they being naturally immune. It destroyed the anthrax bacilli in vitro, and used by injection in susceptible animals, made them immune. It is insoluble in alcohol or water.

Protective Inoculation.—Animals have been rendered immune in various ways—by inoculation of successive attenuated cultures; also with sterilized cultures—that is, cultures containing no bacilli, and with cultures of other bacteria.

Immune Serum.—That obtained from animals rendered immune by attenuated cultures contains protective substances which seem to have some antitoxic action.

Habitat.—In the serum about the wound and in the blood anthrax bacilli are readily found.

The bacillus has never been found free in nature.

CHAPTER XVIII

BACILLUS TUBERCULOSIS AND ALLIED ORGANISMS

THIS very important bacillus was first described, demonstrated, and cultivated by Robert Koch, who made his investigations public before the Physiological Society of Berlin on the twenty-fourth of March, in the year 1882.

Synonyms.—Mycobacterium tuberculosis.

Fig. 47.—Tubercle bacilli in sputum; carbolfuchsin and methylene-blue (Zeiss one-twelfth oil-immersion).

Origin.—In various tuberculous products of man and other animals and in the dust containing the discharges.

Form.—Very slender rods, slightly curved, 2 μ to 4 μ in length, about one-quarter the size of a red blood-corpuscle's diameter, their ends rounded, usually solitary, often, however, lying in pairs in such a manner as to form an acute angle. Sometimes they are S-shaped. In colored prepara-

tions little oval spaces are seen in the rod which resemble spores, but have none of the properties of spores. (See Figs. 47, 48.)

Properties.—Does not possess motility.

Growth.—Requires special media for its growth, and a temperature varying but slightly from 37.5° C. It grows slowly, developing first after ten days, reaching its maximum in three weeks. It is facultative anaërobic. On gelatin it does not form a growth. The media should be slightly acid;

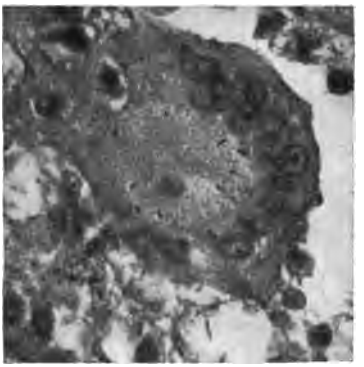


Fig. 48.—Giant-cell containing bacilli (from a photograph made by Dr. Wm. M. Gray).

growth mostly on surface. Subcultures grow more rapidly than those direct from lesions.

Colonies on Blood-serum.—Koch first used blood-serum for culture, and obtained thereon very good growths. Stroke cultures or test-tubes inoculated with small bits of tubercular tissue are placed in a well-ventilated and slightly humid *incubator* at 37° C. for ten to fourteen days, when small glistening white points appear, which then coalesce to form a dry, white, scale-like growth. Under microscope, composed of many fine lines containing the tubercle bacillus.

Glycerin-agar.—By adding 4 to 6 per cent. glycerin to

ordinary agar-peptone medium, Nocard and Roux obtained a culture-medium upon which tubercle bacilli grow much better than upon blood-serum, especially after once obtained in pure culture. Bits of tissue are placed on the surface, not rubbed in until after several weeks; then gently crushed and spread over surface; this hastens growth.

Stroke cultures are used as with blood-serum. They are placed in incubator after inoculation, and remain there about ten days, at a temperature of 37° C. The cotton plugs of the tubes are covered with rubber caps, the cotton first having been passed through the flame, and moistened with a few drops of sublimate solution. The rubber cap prevents the evaporation of the water of condensation, which always forms and keeps the culture from drying up.

The growth which occurs resembles the rugæ of the stomach, and sometimes looks like moistened crumbs of bread. The impression or "Klatsch" preparation shows under the microscope a thick, curled-up center around which threads are wound in all directions. And these fine lines show the bacilli in profusion.

Potato.—It can be cultivated on slices of potato which are placed in air-tight test-tubes to which glycerin has been added.

Bouillon.—Bouillon containing 4 per cent. glycerin is a very good *medium*. Growth on the surface only.

Pure Cultures from Sputum.—Kitasato recommends the thorough washing, changing the water ten times, of the small masses found in the sputum of tuberculous persons. When such specimens are examined, they show tubercle bacilli alone, and when inoculated in agar, give rise to pure cultures.

Animal Inoculation for Diagnosis.—When the bacilli are so few in number in sputum or urine as to make their detection difficult, and also when doubt exists as to the identity of acid-fast bacilli found, several guinea-pigs should be injected in the groin and smears and sections made from the enlarged glands resulting.

Varieties.—Branching and other aberrant forms are not rare, and the tendency now is to class the organism with the

"higher bacteria," mycobacteria, similar to actinomyces. Other acid-fast bacilli exhibit similar types, and it is possible that the bacillary parasitic form is only one stage in the life-history of the organism.

Little granules, arranged like streptococci, which take the characteristic stain, and look as if the protoplasm had been destroyed that inclosed them, are frequently found in sputum. Some believe these "splinters" to develop into regular bacilli in cultures.

Fig. 49.—Tubercle bacillus in sputum (Fränkel and Pfeiffer).

Bovine tubercle bacilli are about one-third smaller than human tubercle bacilli.

Resistance.—Bacilli in sputum, in dark, cool places may live several months. Dried sputum in sunlight and dust is infective not more than ten days. The bacilli will resist in the dry state a temperature of 100° C. one hour. In moisture death occurs at 60° C. in a few minutes.

Chemic Properties.—A waxy substance found in pure cultures, due to fatty acids. The fat-free substance is nucleo-

albumin, and the ash shows a large amount of phosphoric acid. Indol not found.

Staining.—The tubercle bacilli require special methods to stain them, and a great number have been introduced. They are stained with great difficulty, but once stained, they are very resistant to decolorizing agents, hence called *acid-proof* or *acid-fast.* Upon these facts all the methods are founded.

Fig. 50.—Bacillus tuberculosis; glycerin agar-agar culture, several months old (Curtis).

The resisting action of the bacillus to acids is supposed to be due to a peculiar arrangement of the albumin and cellulose of the cell, rather than to any particular capsule around it. A waxy substance, made up of fatty acid, has been found and supposed to account for this resistance. Others believe this substance to be an alcohol.

It will be necessary to describe only those methods principally in use; and as the examination of sputum for bacilli is of so frequent an occurrence and so necessary, it is well to detail in particular the method of staining.

Starting with the sputum, we search for little clumps or rolled-up masses; if these are not present, the most solid portions of the mucus are brought with forceps upon a clean cover-glass; very little suffices. With another cover-glass the mass is pressed and spread out evenly. Drawing one glass over the other, we obtain two specimens, and these are put aside or held high over the flame until dry.

When the preparation is dry and has been fixed by passing through the flame three times, carbolfuchsin is dropped on

the cover-glass and held over the flame until the stain boils; fresh stain is added, the boiling continued for a minute. Then the excess of stain is removed with edge of filter-paper. Decolorize in 25 per cent. nitric or 2 per cent. hydrochloric acid. The excess of acid is then washed out with 95 per cent. alcohol until no further color is imparted to the alcohol, and the smear is gray or light pink in color. The preparation is then washed with water and counterstained with aqueous methylene-blue for ten to thirty seconds.

The Rapid Method (B. Fränkel's Method, Modified by Gabbet).—The principle is to combine with the contrast stain the decolorizing agent; but the preparations are not permanent; the method, however, is very useful.

Two solutions are required: one of Ziehl's carbolfuchsin; the other, Gabbet's acid methylene-blue. (See Formula No. x, on p. 51.)

The cover-glass containing the dried sputum is passed three times through the flame, as described in the general directions. It is then placed in the carbolfuchsin solution five minutes (cold), or two minutes in the hot, immediately transferred to the second solution, the acid blue, where it remains one minute, then washed in water. The preparation is dried between filter-paper and mounted. Examined with oil-immersion.

Slow Method.—The above method may also be used without heating, though in this case a much longer time is required before the bacilli take up the stain. The preparation is left in a small dish or beaker full of carbolfuchsin for eight to ten hours, and then decolorized and counterstained in the way described above. The method is less liable to produce artefacts than the quick method, but is not much used on account of the time it takes.

Examination in Urine.—In urine, owing to the almost inevitable contamination with the smegma bacillus, special methods are necessary to avoid error. The preparation may be left in 97 per cent. alcohol for eight hours, when the smegma bacillus will have become decolorized, or *Pappenheim's*

method may be used: (1) Smear and fix as usual; (2) stain with hot carbolfuchsin for two minutes, pour off the surplus dye without washing; (3) counterstain and decolorize by pouring five times over the preparation the following solution: A 1 per cent. alcoholic solution of corallin is saturated with methylene-blue and 20 parts of glycerin added. Wash in water, dry with blotting-paper, then in the air, and examine. The tubercle bacilli are stained red, smegma bacilli, blue.

Examination of Milk for Tubercle Bacilli.—Place a drop of the sample on a cover-glass and mix it with two drops of a 1 per cent. solution of sodium carbonate. The cover-glass is then gently warmed until evaporation is complete. The saponified fat is then stained, as the ordinary cover-glass preparation. Only a few times has any one succeeded in discovering the bacillus in milk.

Other Acid-fast Bacteria.—The bacillus of leprosy resembles the tubercle bacillus in its staining properties, but gives up the carbolfuchsin more easily and is usually decolorized by the acid and alcohol. It is colored blue by Pappenheim's method.

Acid-fast bacilli have also been obtained from timothy grass, butter, milk, manure, and the surfaces of animal bodies, but differ from the tubercle bacillus in cultural characteristics.

Water has been found to contain acid-fast bacilli; care should be taken to test the water used previously to any important examination for tubercle bacilli.

Biedert's Method of Collecting Bacilli.—When the bacilli are very few in a great quantity of fluid, as urine, pus, abundant mucus, etc., Biedert advises to mix 15 c.c. of the fluid with 75 to 100 c.c. water and a few drops of potassium or sodium hydroxid, then boiling until the solution is quite thin. It is placed in a conical glass for two days, and bacilli with other morphologic elements sink to the bottom of the glass; when the supernatant liquid is decanted, the residue can be easily examined. In this way bacilli were found that had eluded detection examined in the ordinary manner.

The centrifugal machine is used either in connection with Biedert's sediment method or without, to obtain the solids suspended in urine or serum.

Antiformin Method.—A mixture of chlorin water and sodium hydroxid; chlorin is liberated, and this dissolves most of the organisms in the sputum and the mucus, leaving unaltered the tubercle bacillus. Dilute thick sputum with distilled water, add one-quarter volume antiformin, mix until solution is effected; add alcohol, equal volume, and allow mixture to stand eighteen hours. Prepare cover-slip preparations from this.

Staining Bacillus Tuberculosis in Tissue (Sections).—The general method of Gram can be used, but the better way is to use the following:

Warm carbolfuchsin, fifteen to thirty minutes.
5 per cent. sulphuric acid, one minute.
Alcohol, until a light-red tinge appears.
Weak methylene-blue, three to five minutes.
Alcohol, for a few seconds.
Oil of cloves, until cleared.
Canada balsam, to mount in.

Instead of carbolfuchsin, *alcoholic solution of fuchsin* or *anilin-water fuchsin* can be used, but the sections must remain in the stain overnight.

Hardened Sputum and Sectioning.—Sputum can be hardened by placing it in 98 per cent. alcohol. Thin sections can be obtained by imbedding the hardened sputum in celloidin. The sections are then stained as ordinary tissue sections.

To Preserve Sputum.—Sputum can be preserved for future use by placing it in alcohol, where it can be kept for months. Cover-glass preparations can then be made by softening the coagula with a small amount of liquor potassa.

Pathogenesis.—When a guinea-pig has injected into its peritoneal cavity some of the diluted sputum containing tubercle bacilli, it perishes in about three weeks, and the following picture presents itself at the autopsy: at the point of inoculation there is a local tuberculosis—little tuberculous

nodules containing the characteristic bacilli. In the lungs and the lymphatics similar tubercles are found—a general tuberculosis.

If the animal lingers a few weeks longer, the tubercles becomes necrosed in the center and degeneration occurs, the periphery still containing active bacilli, cavities having formed in the center.

Since the bacilli die in course of time, killed by their own products, their number forms no correct guide of the damage present: even their absence in the sputum does not preclude the absence of a tuberculous process. *It is their presence only that warrants a positive declaration.* The number of bacilli in a given specimen is no indication of the severity of the disease.

They are found in the blood only when a vessel has come in direct contact with a tuberculous process through rupture or otherwise. They have been found occasionally in other secretions—milk, urine, etc.

Man is infected as follows:

Through Wounds.—Local tuberculosis.

Through Nutrition.—Milk and meat of tuberculous animals. Phthisical patients swallowing their own sputum and causing an intestinal tuberculosis.

Inhalation.—This is the most usual way, probably constituting the cause in nine-tenths of the cases in adults.

The sputum of phthisical patients expectorated on the floors of dwelling-houses, in handkerchiefs, etc., dries, and the bacilli set free are placed in motion by the wind, or rising with the dust, are thus inhaled by those present. When the sputum is kept from drying by expectoration in vessels containing water, this *great danger* can be *avoided.*

Intra-uterine or placental infection has been demonstrated, but is a great rarity. The ovum or human semen is seldom if ever infected, although tubercular infection of the testicle is common.

Nearly all the cases of supposed *heredity* can be explained as follows: the young children, possessing very little resist-

ance, are constantly exposed to the infection through *inhalation*, and to intestinal infection through milk and other foods.

Immunity.—No one can be said to be immune, though persons who have been greatly weakened offer less resistance than healthy individuals.

Bovine and Human Tuberculosis.—*Tuberculosis in Animals.*—Tuberculosis is probably the most widely disseminated disease among domestic animals, and affects cattle, pigs, horses, dogs, cats, the smaller ruminants, birds, and even turtles and fish. The conclusion of Koch, made public in his address to the Tuberculosis Congress in 1901, that human and bovine tuberculosis are distinct and that infection of human beings from cattle occurs so seldom that no general regulations to restrict it are necessary, has found few adherents. In 1908 Koch reiterated his idea and challenged his opponents to bring proofs to the contrary. Conclusions at this writing seem to be *that 90 per cent. of all pulmonary cases in adult man are not due to bovine infection.* In children under five, however, 10 per cent. of the intestinal tuberculosis and cervical adenitis are due to the bovine type of infection through milk of diseased cows. It is true that certain differences exist between human and bovine tubercle bacilli, the latter appearing to be more virulent to animals, and it is a fact that cattle are very slightly susceptible to the human bacillus, but it is not likely that the converse is so. Children are particularly liable to infection through the gastro-intestinal tract, and it has been shown that the uninjured mucosa of the infant's intestine is permeable to bacilli, so that the pulmonary disease in the young may often be the result of tuberculous bronchial nodes secondary to tuberculous glands of the mesentery.

Various observations on animals have shown that the bacillus occurring in each species has acquired certain special characteristics regarding growth and virulence. The bacilli causing tuberculosis in the cold-blooded animals have departed farthest from the human type, those of birds to a less degree, and those of cattle least of all.

Products of Tubercle Bacilli.—The true nature of the tubercle toxin is not yet clear. It is not unlikely that several toxic bodies differing from one another in their properties are produced. Koch's tuberculin (1890), a bacterioprotein, was obtained by filtering through unglazed porcelain, concentrated glycerin bouillon cultures of tubercle bacilli. It was speedily shown to be devoid of curative power, and is now used mainly for diagnosing the disease in cattle. In healthy animals little or no reaction is produced by the injection of 30 to 40 cg. of tuberculin, but if tuberculous, the temperature rises 2° to 3° F. in eight to twelve hours, and remains elevated for a like period of time and may in larger doses prove fatal. It is dangerous unless used carefully.

Tuberculocidin.—This is an albuminoid obtained from the original tuberculin by precipitation with alcohol. Klebs used it as a treatment for tuberculosis.

Tuberculin residuum, an emulsion from the residuum, hence the name, *T. R.*, is an extract made from dried and powdered living bacilli, and was recommended by Koch in place of the original or old tuberculin, O. T.

Koch's bacillen emulsion (*B. E.*) is similar to tuberculin R, and is a glycerin emulsion of crushed bacteria, this being the entire substance instead of an extract. Theo. Smith recommends virulent uncrushed bacteria killed by moderate heat.

Denys' B. F. (bouillon filtrate) *tuberculin* is a filtrate of liquid cultures to which 0.25 per cent. phenol has been added and allowed to stand two weeks. It is prepared in eight dilutions.

Opsonic Treatment.—In recent years the use of tuberculin R has again been brought forward by Wright and others and curative claims made for it. It is used in very small doses—$\frac{1}{1000}$ milligram at intervals of several days, and the effect on the opsonic index carefully watched.

Use of Tuberculin.—In the use of tuberculin severe reactions are to be avoided. The smallest dose possible is commenced with. *Trudeau* uses for afebrile cases a solution containing $\frac{1}{10000}$ milligram, liquid measure, Koch's B. E., or Denys' B. F.,

increasing 1 decigram of the solution every three days until 1 c.c. of the pure filtrate can be injected without causing any reaction. A negative reaction sometimes occurs in well-advanced cases, and is, therefore, not a proof of the absence of disease. The reaction is due to the stimulation of irritating proteins. Yeast nucleins and other substances have a similar action. This treatment must extend over months. Tuberculin immunity does not last indefinitely. Under this careful treatment, associated with open air, proper food, and general hygiene, Trudeau and his followers have had some very good results.

Von Pirquet Test.—Tuberculin applied to the abraded skin like a vaccination with cow-pox causes a local reaction in tuberculous infants and no reaction in healthy ones. It is not applicable to children over eighteen months of age. The test is so sensitive that it will be positive in the majority of instances, because the majority of people have at some time been affected with tuberculosis or exposed sufficiently to have within them sensitive bodies that are easily stimulated.

Ophthalmic Tuberculin Reaction of Calmette.—A modified form of tuberculin is placed on the conjunctiva of an individual suspected of having tuberculosis. In a few hours a congestion, more or less severe, results, and lasts several days. In healthy persons no reaction occurs. The test is claimed to be harmless, though severe reactions have been reported in tuberculous patients, and even in healthy persons a second application to the same eye may cause an inflammatory reaction.

Moro's Test.—An ointment of tuberculin and lanolin, equal parts, rubbed in the skin of the arm. A crop of papules develops in twelve to twenty-four hours if test is positive.

Agglutination.—Arloing and Courmont have described an agglutination reaction for the tubercle bacillus similar to the Widal reaction of typhoid fever. (See p. 140.) It is very unreliable, however, and but little importance is attached to it.

Antituberculous Serum.—The attempts to produce an effective serum have so far been unsuccessful. Marmorek, by growing the bacillus on a special serum obtained by injecting calves with the leukocytes of guinea-pigs, has secured a toxin which he used to immunize horses, and the serum so obtained has been tried with encouraging results, but its value is still doubtful. Several other sera have been introduced, but none of them has shown any lasting virtues.

Lepra Bacillus (Hansen).—*Origin.*—In 1880 Armauer Hansen declared, as the result of many years' investigation, that he found specific bacillus in all leprous processes.

Fig. 51.—Pure culture of bacillus of leprosy, showing the characteristic morphology and arrangement of the bacilli (Duval).

Form.—Small slender rods, somewhat shorter than tubercle bacilli, otherwise very similar in appearance. Neither in the form nor staining reactions can *B. lepra* be distinguished from *B. tuberculosis.*

In the interior of the cell two or three oval spaces are usually seen, not believed to be spores.

They are immotile.

Growth.—Bordoni-Uffreduzzi have obtained growths upon blood-serum to which peptone and glycerin had been added, but the accuracy of this observation was doubted, and not until Clegg, in 1909, and Duval, in 1910, in work in the Philippine Islands devised special media was it possible to obtain readily initial and subcultures.

The method depends upon supplying the organism with albumin partially metabolized. Clegg prepared this by planting the lepra bacilli on media containing ameba and bacteria; then, by short sterilization, destroying these, while the resistant *B. lepra* lived on. Duval, by adding *trypsin* to

egg-albumin or blood-serum, was able to change the protein sufficiently without requiring ameba or bacterial digestion.

The leprous nodule is cut in small slices and spread over an albumin slant or Petri plate, and the surface covered with 1 per cent. solution trypsin and placed in oven at 37° C. for ten days.

The growth is moist and becomes yellow after several generations; it is on surface.

Staining.—B. lepra resist the decolorizing action of acids, as the tubercle bacilli, but they are more easily stained, requiring but a few minutes more with the ordinary watery solutions. They take Gram's stain readily.

Pathogenesis.—Arning inoculated a prisoner with tissue obtained from leprous patients and produced true leprosy, but this was a susceptible native and the evidence is not clear. Duval, by repeated injections of large amounts of pure culture, has produced leprosy in mice, guinea-pigs, and monkeys.

Rabbits which have been infected through the anterior chamber of the eye showed the lepra nodules (containing the lepra bacilli) diffused through various organs.

In man the skin and peripheral nerves are principally affected, but the lymphatic glands, liver, and spleen can also become the seat of the lepra nodules. The lepra cells which compose these nodules contain the bacilli in large numbers. By applying a vesicant to the leprous skin, the serum thereby obtained will contain great numbers of bacilli. This is a simple diagnostic test.

Method of Infection.—Not yet determined; the air, soil, water, and food of leprous districts have been carefully examined without result. The nasal secretion is very infectious. Intimate contact over a long period seems necessary, but the records of leper asylums show that very few cases ever develop among the attendants.

Smegma Bacillus of Alvarey and Tavel.—Lustgarten, in 1885, through a certain staining process, found peculiar bacilli in syphilitic tissues which he thought had a direct

connection with the disease. But this has been disproved and the cause of syphilis has been found in a protozoön which has been called Spirochæta pallida, which see (p. 209).

The *smegma bacillus* is found in and about the genital organs, is an acid-fast bacillus which resembles the tubercle bacillus in form, but is easily decolorized with alcohol, thus differing from the *latter*. It has no pathogenic properties, but is found at times in the throat and may be mistaken for *B.*

Fig. 52.—Bacillus of glanders from a culture upon glycerin agar-agar (×1000) (Fränkel and Pfeiffer).

tuberculosis. Differentiated in staining, according to Pappenheim's Method.

Bacillus of Glanders (Bacillus Mallei (Löffler-Schütz); Rotz-bacillus).—*Origin.*—In the "farcy buds" or little nodules of the disease, by Löffler and Schütz, in 1882.

Form.—Small slender rods, about the size of the tubercle bacillus. The ends rounded. Never appearing in large collections, usually singly (Fig. 52). Granules like spores appear in some cultures; branched forms are found.

Properties.—They are not motile; do not produce gas; some pigment on *potato.*

Growth.—The growth occurs between 25° and 40° C.—best at 37° C.; it is very sparse upon gelatin, but on glycerin-agar or blood-serum a very abundant growth occurs. Easily destroyed by heat, but cultures sealed and protected from light may live several months.

Colonies.—On agar or glycerin-agar there appear in two to three days small white glistening drops, which under microscope seem as round granular masses with an even periphery, similar to young *B. typhi* colonies.

Stroke Cultures.—On glycerin-agar and blood-serum small transparent drops of whitish or grayish color, which soon coalesce to form a broad band like *B. coli.*

Potato.—An amber-colored, honey-like growth which gradually turns red, then brown, and greenish-brown around it. Weakly acid potatoes are a good medium and give the most typical growth.

Staining.—Gram negative. Since the bacillus is very easily decolorized, some special methods have been recommended. Löffler's and Kühne's solutions for cover-glass and sections. (See *Staining.*)

Pathogenesis.—If horses, field-mice, or guinea-pigs be inoculated subcutaneously with but a very small quantity of culture, a local affection results, followed some time after by a general disturbance; ulcers form at the point of inoculation—little nodules, which then caseate, leaving scars and involving the lymphatics; metastatic abscesses then occur in the spleen and lungs, and death *from exhaustion.* Cattle, pigs, and rabbits are not easily affected; man is readily attacked. The bacilli gain entrance to the blood and urine. Nasal glanders occurs whatever the mode of inoculation. In the horse the type is more chronic than in the mule. A catarrhal nasal discharge occurs, highly infectious. In the cutaneous variety, the enlarged lymphatics or nodes which develop are called *farcy-buds.*

Manner of Infection.—Glanders, being a highly contagious

disease, it requires but a slight wound to allow it to gain entrance.

In horses the primary sore seems to be at the nasal mucous membrane. In man it affects those attending horses and is usually on the fingers, and terminates fatally within three weeks. Chronic glanders may last several years and end in recovery.

Mallein.—A substance called *mallein* has been obtained from the cultures grown in glycerin bouillon. It gives a reaction when injected into cattle suffering from glanders, and is said to be useful in diagnosing the disease. The reaction is specific and never fails to reveal the presence of infection.

The inoculation of a guinea-pig intraperitoneally with some of the suspected discharge will produce an *orchitis* if the glanders bacillus is present, which is quite characteristic and helpful in the diagnosis.

CHAPTER XIX

DIPHTHERIA BACILLUS

Bacillus of Diphtheria (Klebs-Löffler).—*Origin.*—Klebs found it in diphtheritic membrane in 1883; it was isolated by Löffler in 1884.

Form.—Small, slightly curved rods about as long as tubercle bacilli and twice as broad; 1 μ to 6 μ in length; the ends are at times swollen; spores have not been found. Their form is, however, very variable—sometimes much longer than usual, one end often greatly knobbed. Normal bacilli are found only in membrane.

Stained forms are characteristic, since the ends are more easily colored than the center, and usually the bacillus stains in segments, so that it seems to be made up of very short sections or *beaded.* At first sight it appears like a chain of cocci.

Small granules, the Babes-Ernst granules, are shown by the special staining of Neisser.

Properties.—B. diphtheriæ is *immobile; does not liquefy gelatin.* Is not very resistant, being destroyed by a temperature of 50° C., but may live on blood-serum for months. Acid is produced in sugar media.

Growth.—Grow readily on all media, but best on blood-serum mixtures, between temperatures of 20° and 40° C. They are *facultative anaërobic;* they grow quite rapidly and

Fig. 53.—Diphtheria bacilli from a culture on blood-serum, stained by Löffler's methylene-blue solution, showing deeply stained points (×2000) (Wright and Brown).

profusely. Egg cultures (Hueppe's method) give good growths. Passing currents of air increase the growth; on agar, growth is slow.

Colonies on Agar Plates.—At 24° C. little round colonies, white under low power, granular center; irregular borders.

Stab-cultures.—Small, white drops along the needle-track. In glycerin-agar a somewhat profuse growth. Media should be slightly acid.

Potato.—On alkaline surface, a grayish layer in forty-eight hours.

Blood-serum (*After Löffler*).—See p. 69.

In a few hours (eight to sixteen) on the white opaque surface a slight moisture is noticeable which, if examined, is composed of bacilli. In twenty-four hours small round colonies are found which seem to arrange themselves concentrically. The growth becomes more abundant, and the individual colonies larger and yellowish (Fig. 54). On *blood-coagulum* (see p. 72) the growth is usually gray and the margins of the culture crenated. Often a diagnosis can be made in four hours if the serum-tubes are kept in the oven at 37° C. In *milk*, abundant growth, without curdling.

Fig. 54.—Bacillus diphtheriæ; agar-agar culture (photograph by Dr. Henry Koplik).

Bouillon.—In bouillon an abundant growth takes place, and this medium is used to obtain the *toxins*.

Staining.—Is positive by Gram's method. Stained best with Löffler's alkaline methylene-blue. Neisser's double stain (see p. 52) shows granules, blue black, and body, brown.

Pathogenesis.—By inoculation, animals, which naturally are not subject to diphtheria, have had diphtheritic processes develop at the site of infection; hemorrhagic edema then follows, and death.

No agglutinins are developed in the serum.

In rabbits paralyses develop, and when the inoculation occurs upon the trachea, all the prominent symptoms of diphtheria show themselves.

Manner of Infection in Man.—The exact way is not yet known. It is supposed that the mucous membrane, altered in

some manner, the diphtheria bacillus then gains entrance and the disease develops. The bacilli may be found in healthy individuals who may act as a source of infection to susceptible individuals without themselves becoming infected. They are seldom found in blood or other tissues; the symptoms arise mainly from the absorption of the toxin.

Prevalence of Bacillus Diphtheriæ.—Examinations made on a large scale of the throats of supposedly healthy individuals have shown that the Bacillus diphtheriæ is rather widely distributed. Not only does it linger for many weeks in the throats of persons recently recovered from the disease, but it

Fig. 55.—Bacillus diphtheriæ, from a pure culture.

is found in the caretakers, nurses, etc., and there are allied organisms, with more or less pathogenicity, that have been found in atrophic rhinitis, in conjunctivitis, and in the throats of unexposed normal individuals.

Pseudodiphtheria.—The *pseudobacillus of Hoffman* is believed by some investigators to be but a weakened diphtheria bacillus that has lost its toxic power, but its true relation is not settled. It is morphologically identical and at times is found side by side with the true bacillus. It grows well on agar, shows no granules with *Neisser* stain, and, contrary to *B. diphtheriæ*, does not produce as much acid in dextrose broth.

Methods of Diagnosis.—A small piece of exudate or some secretion from pharynx, tonsil, or nares is obtained on a sterile cotton swab and transferred, as soon as possible, to the surface of two or more blood-serum tubes (if these are not available, the swab should be placed in a sterile test-tube or bottle, and sent to the laboratory at once). The inoculated tubes are placed in the incubator at 37° C., and examined in twelve hours. If a growth is visible, a slide is made and stained

Fig. 56.—Bacillus diphtheriæ, from a culture upon blood-serum (×1000) (Fränkel and Pfeiffer).

with Löffler's and Neisser's stain, and if bacilli are present, with characteristic granules, the diagnosis of diphtheria is most probable. *Negative results* are not to be depended on. The use of antiseptics, gargles or the failure to obtain a portion of the exudate may give a negative culture result in a case of diphtheria. If the symptoms are suggestive, it is best to use antitoxin and isolate the patient, notwithstanding a negative report from the laboratory. If there are no clinical signs, the growth should be tested for toxicity by inoculating a

guinea-pig; it should be grown in alkaline sugar bouillon and tested in two days for acid. The xerosis and Hoffman's bacilli are not pathogenic for guinea-pigs.

Products.—But it is not the mere presence of the bacillus that gives rise to trouble: certain products which generate it get into the system and produce the severe constitutional symptoms.

Toxins of Diphtheria.—Roux and Yersin, in 1888, discovered the toxin and showed that the injection of the filtered culture bouillon (that is, freed of all diphtheria bacilli) gave rise to the same palsies as when the bacilli themselves were introduced.

The toxins may be separated from three-weeks-old bouillon cultures by filtration. They are not albumins and are very complex. Ehrlich claims three forms: one he calls *toxone;* the other, *toxin;* the toxone produces paralytic symptoms and appears to be less affected by antitoxin; the third, *toxoid,* combines with antitoxin. The toxins are highly poisonous—0.001 c.c. may be sufficient to kill a guinea-pig in less than twenty-four hours. The substance is unstable, losing its toxic power gradually. Heating at 58° C. for two hours is destructive, but drying renders it more stable. Direct sunlight destroys its power in a few hours. Boiling in *five minutes.* If kept cold and in the dark, it may remain active two years. Alcohol and calcium chlorid precipitate the toxic element.

Antitoxin.—Behring, in 1890, found that animals rendered immune had a principle in their blood that was antagonistic to the development of the toxin.

Immunity.—Brieger and Fränkel, by injecting 10 to 20 c.c. of a three-weeks-old culture of diphtheria bacilli which had been heated at 70° C. for one hour, produced an immunity in guinea-pigs against the virulent form. This active principle is unknown chemically, but has been called *antitoxin.*

The toxin generated by the germ is supposed to be neutralized by the antitoxin and prevented from injuring the body tissues. The value of antitoxin in diphtheria seems to be established beyond a doubt, and it is the claim of eminent

sanitarians that the death-rate from this disease has been reduced from 66 per 100,000 to 19 per 100,000 since the use of antitoxin (Park).

The strength commonly employed in human beings is 5000 units, and as much as 120,000 units may be given without detriment in severe cases. If this amount is injected subcutaneously and even intravenously into a child suffering from diphtheria in the earlier stages (second to third day), the disease is often arrested. The membrane begins to disappear, and in two or three days has vanished. The constitutional symptoms are likewise greatly influenced by the injection. For prophylaxis and immunizing well persons 1000 to 3000 units are employed.

In such conditions as asthma severe and fatal results have followed the use of the serum, and some cases of peculiar sensitiveness to horse serum (see *Anaphylaxis*) have been reported, fatal results having occurred, but fortunately such mishaps are exceedingly rare.

The *antitoxin* has no influence on the bacteria themselves; their virulence and length of residence in the body are not lessened.

Preparation of Antitoxic Serum.—Horses are rendered immune by gradually increased doses of diphtheria toxin, the power of the toxin having first been standardized by its neutralization with some standard antitoxin in powdered form.

Preparation of Toxin.—The bacillus is grown in veal broth with an alkaline reaction. (Acids prevent toxin formation.) There should be a free supply of oxygen, and, therefore, large shallow flasks are used. The maximum toxicity is developed in seven to ten days. The strength should be $\frac{1}{500}$ c.c., fatal for 500-gram guinea-pig.

The toxin is at first injected subcutaneously, then intravenously, and after several months' treatment a resistance is obtained that will withstand 300 to 500 times the original lethal dose. The horse is then bled, and from five to nine liters withdrawn; this is then allowed to coagulate, and under very careful precautions the serum is placed in sterile packages,

its strength having first been compared with a standard furnished by the United States Government. *Unless kept in the dark* and at low temperature, it loses strength rapidly.

Antitoxic Unit.—An *immunity unit*, according to Ehrlich, is the amount of antitoxic serum which will neutralize 100 times the minimum lethal dose of toxin, when serum and toxin mixed and injected into a 250-gram guinea-pig does not cause death in four days. Thus, if the serum will protect in doses of $\frac{1}{50}$ c.c., then each cubic centimeter has 50 units' power, and 20 c.c. will contain 1000 units, or will be sufficient to neutralize an amount of toxin that would be fatal for 25,000 kilos (12,500 pounds) of guinea-pigs, or 100,000 pigs weighing 250 grams each. The serum is concentrated by precipitation and separation from the blood-serum of the pseudo-globulins containing the antitoxic principle, so that 10 c.c. contain more units than formerly. The doses given now are much larger than when first introduced. As much as 100,000 units have been employed in a single case. (Sera containing 1000 units to 1 c.c. are now being marketed.)

Streptococcus in Diphtheria.—Streptococci have been found quite constant in diphtheria, but they resemble the Streptococcus pyogenes, and have no specific action.

CHAPTER XX

THE COLON-TYPHOID GROUP

IN this group are placed a variety of organisms similar in form and growth and having many biologic properties in common, but differing in pathogenesis. The more important members of this group are: *Bacillus coli, B. typhosus, B. enteritidis, B. dysenteriæ.* Another closely related organism is the *B. suipestifer* (hog cholera). The form is usually a plump rod with rounded ends. Gram-negative. No spores.

Motile, all possessing flagella, on gelatin surface, a leaf-shaped, thin colony. They all reduce nitrate to nitrite. Gelatin not liquefied. Ferment sugar broth; some produce acid in milk, some do not. Some form gas in sugar, some not.

Bacillus Coli (Escherich).—*Synonyms.—Bacterium coli commune; Colon Bacillus.*—Found (1886) in human feces, intestinal canal of most animals, in pus and water.

Form.—Short rods, with very slow movement, often associated in little masses, resembling the typhoid germ, flagel-

Fig. 57.—Bacillus coli communis, from an agar-agar culture (× 1000) (Itzerott and Niemann).

lated, not forming spores (Fig. 57). Very short round ends; oval forms are found in animal tissues.

Properties.—Does not liquefy gelatin, causes fermentation in saccharine (dextrose) solutions in the absence of oxygen, forming *gas*. Two parts hydrogen to 1 part carbon dioxid. Produces acid fermentation in milk; coagulates; its optimum temperature for growth is 37° C.; causes formation of indol in peptone solutions. In bouillon, forms cloudiness with slimy precipitate. Some cultures non-motile.

Growth.—On potato a thick, moist, yellow-colored growth; on agar a gray-white growth; on gelatin a growth similar to typhoid. It can also develop on phenol-gelatin, and withstands a temperature of 45° C.

Staining.—Ordinary stains; does not take Gram.

Pathogenesis.—Inoculated into rabbits or guinea-pigs, death follows in from one to three days, the symptoms being those of diarrhea and coma; after death tumefactions of Peyer's patches and other parts of the intestine; perforations into peritoneal cavity, the blood containing a large number of bacilli.

The colon bacillus by many writers is held responsible for most of the complications of typhoid fever, such as peritonitis, cholangitis, etc.

Epidemics of a cholera or dysentery nature, called by Escherich *colitis contagiosa*, and due to infection of water and food, have been noted by a number of writers. The onset is very sudden and prostrating, though not fatal.

Many other forms of suppuration are associated with the presence of Bacillus coli.

It is supposed to give rise to cystitis, infecting the bladder either through the urethra or the blood. The urine is then acid.

Distribution.—The bacillus has been found very constant in acute peritonitis and in cholera nostras. All normal persons harbor the *B. coli* in the intestine, where, under ordinary conditions, it produces no disturbance. After death it multiplies rapidly, invading the tissues.

In Water.—The presence of *B. coli* in surface waters is natural, owing to contamination with the fecal discharges of man and other animals. In well-water its presence denotes sewage or surface contamination, and such a well should be condemned until free from *coli.* (See Water Analysis.)

Bacillus of Typhoid or Enteric Fever (Eberth-Gaffky).—*Origin.*—Eberth, in the year 1880, found this bacillus in the spleen and lymphatic glands of persons dying of typhoid, and Gaffky isolated and cultivated the organism four years later.

Form.—Rods with rounded ends about three times as long as they are broad. Usually solitary in tissue-sections, but in old artificial cultures found in long threads. Flagella on all sides (Fig. 58).

Properties.—Very motile. *Spores* have not been found; they do not liquefy gelatin.

Growth.—They are facultative anaërobic; grow best at 37° C., but can also develop at ordinary room temperature. They develop chiefly on the surface, and very slowly. Repeated

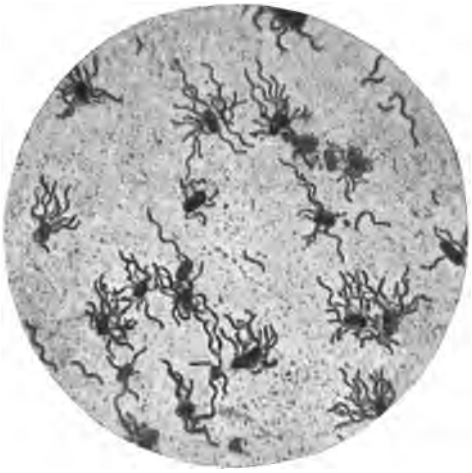


Fig. 58.—Bacillus typhi, from an agar-agar culture six hours old, showing the flagella stained by Löffler's method (×1000) (Fränkel and Pfeiffer).

freezing and thawing do not affect the vitality of the germ, and phenol in 1 to 2 per cent. solution has no effect on it. A ten-minute exposure to 60° C. is invariably fatal.

Colonies on Gelatin Plates.—Two forms: the ones near the surface spread out like a leaf, transparent, with bluish fluorescence. The deeper ones resemble whetstone crystals of uric acid, with the same yellowish tinge (Fig. 59).

In five days they attain to 3 millimeters in diameter.

Bile Salt Media.—Rapid growth without gas formation; a number of special media suited for the growth of typhoid, namely, Jackson's, Hesse, Hiss, Conradi-Drigalski, etc. (See Water Analysis and formula for Media.)

Stab-cultures.—Mainly on the surface, a pearly layer.

Agar Stroke Cultures.—A transparent thick layer.

Potato.—The growth here is quite characteristic. At 37° C. in forty-eight hours a moist, transparent film is formed over the whole surface, but so transparent that it can hardly be seen without close observation. If a small portion of this is placed under a microscope, it will be seen swarming with bacilli (Fig. 60).

The growth never becomes more prominent; the potato must have a neutral or acid reaction.

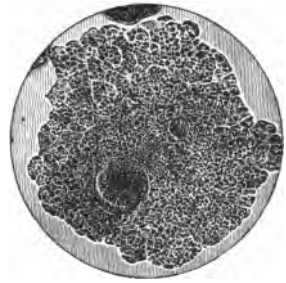


Fig. 59.—Colonies of typhoid bacilli three days old (×100) (Fränkel and Pfeiffer).

On Potato Gelatin.—The colonies do not have the yellow color; they are transparent; later on they become dark brown with green iridescence.

Milk.—The bacteria grow very well in milk, producing a slightly acid reaction, but no *coagulation.*

Fermentation Tube.—In *sugar broth,* in the fermentation tube, *acids are formed without gas.*

Glucose Gelatin.—In glucose gelatin there is no gas-production. Indol is likewise not generated by the typhoid bacillus, whereas it is by the colon bacillus.

Staining.—Colored with the ordinary anilin dyes, when they are *warmed;* since they are easily decolorized, acids should be avoided. Gram negative.

Distribution.—Outside of the body it is rarely found. Typhoid or enteric fever is a general infection, but affecting chiefly the Peyer's patches of the intestine. The bacilli are found in the intestinal glands and in the enlarged and deeply

congested spleen. Metastatic abscesses form in various parts of the body, and here likewise the organisms abound. They occur in the feces only in small numbers, more commonly in the urine. The urine may contain active bacilli for weeks after recovery from the fever.

Typhoid Bacilli in Water.—Although all evidence shows that the water-supply is a frequent source of infection, very few persons have ever isolated the typhoid bacillus from such

Fig. 60.—Bacillus typhosus. Impression preparation from gelatin plate. Fuchsin (×1000) (Hicks).

an infected source. The earlier reports show that no account was taken of *Bacillus coli*, which is usually present in polluted waters. (See Water Analysis.)

Persistence in Water.—Franckland kept bacilli alive in water, sterilized by heat, seventy-five days; in filtered water at 19° C., five days; at 6° C., twelve days. In ordinary water they are likely to be destroyed in a short time by the overgrowth of other bacteria. Under ordinary conditions they do not multiply, but decrease steadily in numbers. In soil

they are more persistent. *Sewer-gas* or *air* is never a source of *infection.*

Mode of Infection.—The bacilli in the dejecta of the diseased person find their way into drinking-water, milk, or dirty clothes, and so into the alimentary tract of a person predisposed to the disease. *Flies* act as conveyors by infecting food. The bacilli enter the blood through the lymphatics, and so become lodged in various organs. They are quite resistant, living for some time in the soil and water, and are more resistant than other organisms to the action of phenol. An epidemic has been traced to the eating of oysters taken from contaminated water. Milk-cans washed in polluted water may be the origin of an epidemic. Ice is rarely a cause.

Pathogenesis.—Lower animals do not have enteric fever, though their death has been caused by injection of the bacilli into the veins of the ear and peritoneum due to toxic substance.

In man the bacillus has been found in the urine, blood, sputum, milk, intestinal discharges, roseolar spots, and in various organs, as spleen, liver, lymphatic glands, and intestinal villi.

It is found in secretions several days after the attack has subsided. It is found in this disease only.

Typhoid Carriers.—Some individuals retain a culture of the bacilli in the gall-bladder for years, and manufacture, or at least expel, true virulent bacilli through the feces and urine intermittently. Such persons have infected other individuals without suffering any inconvenience themselves. Some forms of chronic inflammation, as cholecystitis and appendicitis, have been caused by the typhoid bacillus, though more often the colon bacillus is found.

Products.—Brieger found a substance in the cultures, which he named *typhotoxin*, with the formula $C_9H_{17}NO_2$. It has no specific action. A toxalbumin insoluble in water has also been isolated, but, as experiment animals are immune to the disease, no definite actions have yet been determined.

The cultures, when old, show an acid reaction.

Antityphoid bacterins (vaccines) have been used very extensively in armies and institutions as a prophylactic or pro-

tective. *Bacterins* are made from a weakly virulent culture. The results so far obtained would indicate that this inoculation has some value, but the evidence is far from conclusive and the statistics on the subject require more careful study before they can be accepted as positive proof. An eighteen-hour agar surface growth is washed in sterile salt solution and killed by heating at 56° C. one hour. It is then diluted so as to contain one billion bacilli to 1 c.c. Tricresol, 0.25 per cent., is added to preserve, and animals tested for purity of the vaccine. A slight local reaction follows the inoculation; about three injections of ½, 1, and one billion bacilli at ten-day intervals, render the subject immune. General symptoms rarely occur.

The Gruber-Widal Blood-serum Test.—In 1896 Widal and Grünbaum, working separately, developed what is now spoken of as the "Widal serum test," or "*Widal reaction*," or *agglutination test.* It consists in testing a drop of blood of a patient suspected of having typhoid fever, by mixing a dilution of it with a drop of a fresh bouillon culture of typhoid bacilli, and examining the mixture in a hanging drop under the microscope. Within fifteen minutes to an hour the motility of the bacilli will cease, and they will have arranged themselves into clusters, as if stuck or glued together (Fig. 61). If this reaction occurs within an hour, and with the proper dilution of the serum, the patient has or has had typhoid fever. Widal first used the serum of the blood; this has been modified so that a drop of dried blood is sufficient.

Method of Test.—The method as applied in city laboratories is as follows: The physician is told to clean the finger of the patient with water (no germicides), and with a needle draw a drop of blood on to a piece of ordinary note-paper. This is then sent to the laboratory; the paper with the dried blood is soaked for a few minutes in a watch-glass containing 4 drops of clean water, thus obtaining a dilution of 1 : 5. One drop of this is then mixed with one drop of a bouillon culture of typhoid bacilli of about twenty-four-hours' growth, and examined under the microscope in the hanging drop. Weaker

dilutions of the serum have been recommended (1 : 50), and this should be used in cases of doubt. So far, about 95 per cent. of the cases examined, and which clinically were considered typhoid fever, have given a positive reaction. It is not often present until the fifth day of the fever, and disappears usually within a year, though in some individuals it has been found ten years after an attack of the disease.

The agglutinating properties have been found in nearly all the secretions of the body—tears, urine, milk, pleuritic effusions serous fluid from blisters, etc.

Fig. 61.—The Widai agglutination reaction (Slater and Spitta).

There is no relation between the reaction and the bactericidal power of the serum; the agglutination is not a destruction. The agglutinating power is active, though the blood be dried and sealed up for months. It seems to have no direct relation with the question of immunity, since it occurs at the height of the disease, and intense agglutinating serum may be had in severe cases and in cases with relapses. A negative result does not exclude typhoid.

The test is *quantitative—i. e.*, it depends upon the dilution of the blood-serum, since the serum of healthy persons in strong dilution will cause agglutination and loss of motility.

A serum in a dilution of 1 : 100 causing complete clumping in half an hour is undoubtedly typhoid.

The culture must be kept in a vigorous condition by frequent subplanting, and must be tested occasionally with normal serum. Cultures kept in an incubator for a long time tend to agglutinate spontaneously.

Macroscopic Agglutination Test.—Where laboratory facilities are not available, the sedimentation test is practical. It consists in adding the diluted blood or serum to be tested to a suspension of dead typhoid bacilli in salt solution. If the reaction is positive, a flocculent precipitate forms which consists of masses of agglutinated bacilli. A control tube containing normal serum and the suspension should remain opaque and show no flocculi.

Differentiation Between Colon and Typhoid.—The colon bacillus and the typhoid bacillus resemble each other so closely that much attention has been paid to methods of differentiation.

Points of Resemblance Between Bacillus Typhi and Bacillus Coli Communis.—First, microscopic appearance; second, agar and gelatin cultures; third, sometimes growth on potato the same; fourth, staining peculiarities; fifth, resistance to phenol.

Points of Difference:

Colon Bacillus.	*Typhoid Bacillus.*
Bile media, gas.	None.
Less motile.	Actively motile.
Gelatin colonies develop more rapidly.	Develop slowly.
Produces gas on dextrose or lactose media.	Does not.
Coagulates milk.	Does not.
Produces indol.	Does not.
Growth on potato visible.	Invisible.
Changes neutral red to yellow.	Does not reduce neutral red.
Endo-fuchsin red.	Not.

Differences are also noted in the growth on special media, such as those of Hiss and Elsner. On *Elsner's potato-gelatin* the colon bacillus and the typhoid bacillus both grow readily. The *medium of Hiss* is of some assistance in isolating the germ. (See p. 75.)

Hiss Media.—Shows *B. coli*, large colony, even borders. *B. typhi*, small colony, hairy and fringy threads. (See p. 75.)

Endo-fuchsin-lactose Agar (see p. 77).—Incubation on plates of this media shows *B. coli* red; typhoid as clear, colorless drops.

Malachite green added to agar permits the growth of *B. typhi*, but not *B. coli*. The dye must be as nearly neutral as possible.

Bile Salt Media.—Fresh bile or sodium taurocholate added to lactose glucose agar or broth permits the rapid growth of both *B. typhi* and *B. coli*, fermentation with gas formation denoting *B. coli*, growth without fermentation meaning typhoid.

Drigalski and Conradi Media.—Petri plates filled with this media are inoculated on the surface only. Placed in incubator sixteen to twenty-four hours. Typhoid colonies small, transparent, and blue. Colon colonies red, coarser, and larger.

Typhoid Bacilli from Blood.—Conradi, Busquet, Coleman and Buxton, and others have found the bacilli in the blood of every patient by the following method: A mixture of ox-bile, 90 c.c., glycerin, 10 c.c., and peptone, 2 gm., is distributed into 20 c.c. flasks and sterilized. Ten cubic centimeters of blood is drawn from the elbow into a glass syringe and divided among three flasks. These are incubated, and in twenty-four hours litmus-lactose-agar plates are inoculated on the surface by a stroke from the flasks. A growth is obtained in five or six hours.

If the growth is a bacillus which has not reddened the medium, it is tested for the Widal reaction with immune serum. The diagnosis has been made as early as the second day.

Paracolon or **paratyphoid bacilli** are members of the

colon group described by Widal, Gwyn, Schottmüller, and others. They are of importance, since they produce fevers clinically resembling a mild form of typhoid, but which are rarely fatal. They may be the sole cause of the disease, and also occur together with the typhoid bacillus in mixed and secondary infections. Morphologically, they resemble the typhoid bacillus, but differ from it culturally and give their own serum reactions with the blood of affected patients. They ferment glucose, but not lactose or saccharose; litmus milk at first becomes acid, but later grows alkaline and is not

Fig. 62.—Bacillus botulinus, with spores. Pure culture on sugar-gelatin. Van Ermengem prep. (Kolle and Wassermann).

coagulated. On potato a slight visible growth occurs; indol is usually not formed. Typhoid sera do not agglutinate paracolon bacilli, and vice versâ; also different paracolon infections may not agglutinate each other.

Bacillus Botulinus (Van Ermengem).—An anaërobic bacillus cultivated by Van Ermengem in 1896 from ham which had caused poisoning.

Form.—A large bacillus with rounded or spindle-shaped ends, and often with oval terminal spores, motile, with lateral flagella (Fig. 62).

Staining.—Gram positive, easily stained with ordinary dyes.

Growth.—Strictly anaërobic. Forms abundant gas in glucose, gelatin, and liquefies cultures, producing butyric acid odor. Best temperature between 20° and 30° C.

Pathogenesis.—Produces a powerful toxin in the tissues, like the tetanus bacillus. This toxin may be present in the affected meat without causing decomposition, and thus give rise to poisoning.

Bacillus Dysenteriæ (Shiga, 1898).—The term dysentery is applied to an intestinal disease displaying more or less

Fig. 63.—Bacillus dysenteriæ from agar culture. Fuchsin stain. Zettnow prep. (Kolle and Wassermann).

constancy in its clinical manifestations, but having, as is now known, a variety of causative agents. It is fairly certain that one type is the result of infection with an ameba, while non-amebic forms can probably be produced by several bacteria. Chief among these is the bacillus first described by Shiga in Japan, and since then found by Kruse in Germany, by Flexner, Strong, and Harvie in the Philippine Islands, and by Vedder and Duval in the United States. The fact that it is constantly present in the feces in one type of dysentery, that

garis in that the gelatin is less rapidly liquefied. Found also in putrid material.

Proteus Zenkeri (Hauser).—Does not liquefy gelatin; otherwise similar to the other two.

CHAPTER XXI

CHOLERA BACTERIA

Spirillum Choleræ (Koch) (Comma Bacillus of Cholera).—*Synonym, Vibrio Choleræ.*—*Origin.*—Koch, as a member of the German expedition sent to India in 1883 to study cholera, found this microorganism in the intestinal contents of cholera patients, and by further experiments identified it with the disease.

Fig. 64.—Comma bacillus, pure culture (× 600).

Form.—The spirillum as seen ordinarily appears as a short, arc-like body, about half the size of a tubercle bacillus, but when seen in large groups, spirals are formed, each little arc appearing then as but a segment, *a vibrio.* Each arc is about three times as long as it is broad, and possesses a flagellum at one end. Old agar cultures show straight forms; **S**-shaped forms not uncommon, made of two vibrios end to end (Fig. 64).

Properties.—The spirilla are very motile; liquefy gelatin. They are easily affected by heat and dryness. Spores have not been found.

Growth.—At ordinary temperatures on all nutrient media that have an alkaline or neutral reaction. Strongly aërobic.

Colonies, Gelatin.—After twenty-four hours, small white points which gradually come to the surface, the gelatin being

slowly liquefied, a funnel-shaped cavity formed, holding the colony in its narrow part, at the bottom, and on the fifth day all the gelatin is liquid. If the colonies of three days' growth are placed under microscope, they appear as if composed of small bits of frosted glass with sharp, irregular points.

Stab-culture.—After thirty hours a growth can be distinguished along the needle-track, and on the surface a little cavity is formed, filled by a bubble of air, and this liquefaction

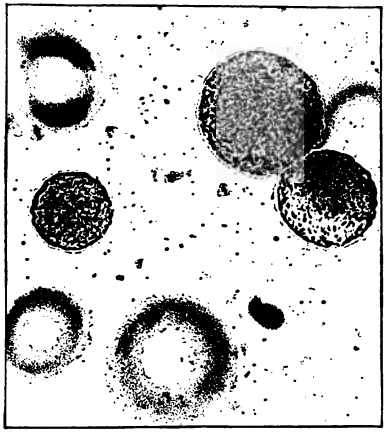


Fig. 65.—Cholera colonies after thirty hours (×100) (Fränkel and Pfeiffer).

proceeds until, on the sixth day, it has reached the sides of the tube, tapering, funnel-shaped, to the bottom of the tube. After several weeks the spirilla are found in little collections at the bottom of the fluid gelatin. In eight weeks the bacilli have perished.

Agar.—Stroke cultures. A shiny white layer which lasts many months.

Alkaline Agar.—Plates at 37° C. Flat discs, transparent, grayish blue.

Potato.—A yellow, honey-like, transparent layer if the potato is kept at animal heat.

Bouillon.—A wrinkled scum is soon formed in bouillon. The spirilla live well and grow in sterilized milk and sterilized water, remaining virulent in the latter for many months.

Fig. 66.—Cholera bacillus (forty-eight hours; 5 per cent. gelatin).

Fig. 67.—Cholera bacillus (sixty hours; 5 per cent. gelatin).

Fig. 68.—Cholera bacillus (seventy-two hours; 15 per cent. gelatin).

Figs. 66–68.—Tube-cultures (from United States Government Report on Cholera.—Shakespeare).

In ordinary water the bacteria present are destructive to the comma bacilli, and they die in a few days.

Dunham's Peptone Solution.—Useful for the development of nitrites and the indol reaction. (See p. 77.) Also for the rapid development of the cholera vibrio. In four hours after inoculation of peptone water pure cultures may be obtained;

best to make several plantings from the peptone to agar after six hours' growth.

Dieudonné's Medium.—(See p. 77.) In this cholera vibrio grow abundantly; other intestinal bacteria very scantily. This medium valuable mostly for feces, less for infected water.

Staining.—They are colored well with watery anilin solutions. The flagella can be well seen by staining according to the flagella stain or *Giemsa.*

Pathogenesis.—Experiment animals are not subject to cholera Asiatica, but, by overcoming two obstacles, Koch produced choleraic symptoms in guinea-pigs. Nicati and Rietsch prevented peristalsis and avoided the acidity of the stomach-juices by direct injection into the duodenum, after tying the gall-duct. Koch alkalinized the gastric juice with 5 c.c. of 5 per cent. solution of sodium carbonate, and then injected 2 grams of opium tincture for every 300 grams of weight into the peritoneal cavity, paralyzing peristalsis. The cholera culture then introduced through a stomach-tube, the animals die in forty-eight hours, presenting the same symptoms in the appearance of the intestines as in man, the serous effusion containing great numbers of spirilla. *Rabbits* injected into the ear veins with cholera cultures die very quickly and present intestinal lesions. The vibrio is met with in the layer of flaky mucus which coats the surface of the intestine.

It may invade the biliary passages.

Manner of Infection in Man.—Usually through the alimentary tract, with the food or drink, the intestinal discharges of cholera patients having found entrance into the source of drinking-water. Soiled clothes to fingers, fingers to the mouth, etc.; torpid catarrhal affection of the digestive tract predisposing. The spirilla are not found in the blood or any organ other than the intestines—the tissue of the small intestines. They are also found in the vomit and the intestinal contents.

Toxins.—From broth cultures soluble toxins which have a hemolytic action have been isolated. The toxin is easily destroyed by heat (thermolabile).

Products—"*Cholera red.*" *Indol Reaction.*—Present in peptone water cultures containing nitrates. The indol is shown by the addition of a few drops of pure sulphuric acid, the solution turning *red*—the so-called "*cholera red.*" Once thought distinctive, but other bacteria also give rise to *indol*, and the same reaction.

Serum Agglutination Test.—The agglutination test is made in the same way as the Widal test for typhoid fever. Agglutinins appear in the blood five to ten days after infection.

Fig. 67.—Comma bacillus in mucus, from a case of Asiatic cholera.

Cultures in serum dilutions of 1:1000 up to 1:10,000 are agglutinated.

Detection of Cholera Organisms in Drinking-water.—When a few bacteria are supposed to be present in fecal matter or drinking-water, it is best to add a large quantity of the material (200 c.c. of drinking-water) to about 10 c.c. of bouillon or peptone-water, and place the mixture for twenty-four hours in an incubator, which will cause rapid reproduction, and then the organisms can be readily discovered.

From Feces.—The following technic is recommended:

1. Examine mucous flakes in stained preparations and hanging drop.

2. Isolate on agar media at 37° C.

(a) Plant plates of alkalinized agar and Dieudonné's medium with particles of feces.

(b) Plant in 50 c.c. peptone solution 1 c.c. fecal matter. After six hours or longer in the incubator at 37° C. take several loopsful from the surface and plant on several plates Dieudonné's and ordinary alkaline agar.

(c) Investigate agglutination reaction, using drops from isolated colonies and secure pure cultures.

3. Demonstrate the reaction of Pfeiffer and agglutination with the pure colonies.

Protective Bacterins.—Virulent cultures killed by heat have shown protective power and were used extensively during an epidemic in Japan.

Haffkine has obtained a great reduction in mortality in cholera regions by the use of anticholera bacterins as a protective measure.

Serum therapy has not been successful.

Carriers.—In some recent examinations of persons exposed to cholera, carriers of typical cholera vibrio have been found. The vibrio may be found, as in typhoid fever, in the gall-bladder.

Pfeiffer's Reaction and Agglutination.—The serum of an animal (a rabbit) made immune against cholera by the injection of sterile or living cultures, intravenously, three times at intervals of a week, has an action against the cholera spirillum. It first precipitates the bacteria out of an emulsion, leaving a clear liquid (agglutination), and then dissolves the bacteria (bacteriolytic action), leaving only spheric granules. This action is specific, *i. e.*, the cholera immune sera will affect only cholera vibrio. Such serum is not antitoxic, *it is bacteriolytic.* For diagnostic purposes an agglutination in dilution of 1 : 1000 by a serum with an activity of 1 : 4000 is suspicious of cholera. The blood-serum of convalescents

and cholera-vaccinated individuals contains the same bactericidal substances.

Allied Varieties.—Many vibrios resembling the spirillum of cholera have been isolated from drinking-waters and from the stools of persons suffering with diarrhea, and some bacteriologists are inclined to consider them as varieties of the true cholera spirillum, which under certain conditions become pathogenic. Among these are Spirillum berolinense, S. dunbarii, S. danubicum, S. of Wernicke, S. bonhoffii, S. weibeli, S. schuylkilliensis, S. milleri, S. aquatilis. The last two are non-pathogenic for experiment animals, also the Finkler-Prior vibrio, vibrio Metchnikovii, and tyrogenum, which have historic interest because of their close identity with the cholera organism, but with the agglutination tests and Pfeiffer phenomenon they have been shown to be dissimilar.

Conclusions of International Committee of Public Hygiene, Adopted October 9, 1911.—Every choleriform vibrio can be considered as truly choleraic which presents agglutination in the proportion of at least 1:1000 by a cholera serum of 1:4000 activity, or a positive Pfeiffer reaction, and every choleriform affection in which is encountered such a vibrio should be considered as a case of cholera.

Method of Pfeiffer.—1. Secure immune serum by injecting into peritoneal cavity of a *rabbit* an entire agar culture which has been killed by heating for one hour at 56° C. Fourteen days after collect the blood-serum.

2. Dilute the suspected vibrio by adding one loopful of an eighteen-hour-old agar culture to 1 c.c. meat water.

3. Add to the above about 1 milligram of immune serum and inject this into peritoneal cavity of a guinea-pig.

4. At the same time a second guinea-pig is inoculated with diluted culture (2), but without the serum.

5. A third guinea-pig is inoculated with a similar dilution of culture to which has been added about 10 milligrams normal rabbit serum.

6. At the end of twenty minutes, and again at the end of one hour, some of the peritoneal fluid is examined from each

pig, under strong magnification, in hanging drop and dark field illumination.

7. The reaction is positive if in the fluid from No. 1 pig the vibrios are dissolved, while in that from No. 2 and No. 3 the vibrios are very motile and active and form well preserved.

It is necessary that the vibrio be of good virulence.

Method of Bordet.—As experiment animals are not always available, Bordet has elaborated a test-tube method. The immune serum is diluted 1:50, 1:100, 1:500, and 1:1000. Into a series of test-tubes there are poured 5 drops of a guinea-pig serum, 5 drops of a mixture of suspected culture (one loopful of an eighteen-hour-old agar culture to 1 c.c. salt solution), and enough of immune serum and salt solution to make the necessary dilution and up to 20 drops. A series of controls is made with *normal serum* and the same amount of microbic culture and guinea-pig serum.

After eighteen hours the cholera vibrios will be active in the control, but dissolved and clumped up in the tubes containing the immune serum.

CHAPTER XXII

BACTERIA IN PNEUMONIA

KLEBS in 1875 called attention to the presence of bacteria in pneumonia, and in 1882 Friedländer developed a bacillus from the lung tissue of a pneumonic person which he thought was a coccus, and called it pneumococcus.

In 1886 A. Fränkel and Weichselbaum proved that this organism was not constant—in fact, was rare.

A. Fränkel obtained in the majority of cases of pneumonia an organism that he had described in 1884 under the name of sputum-septicemia micrococcus.

Weichselbaum called this *Diplococcus pneumoniæ*, and be-

Fig. 70.—Bacillus pneumoniæ of Friedländer, from the expectoration of a pneumonia patient (×1000) (Fränkel and Pfeiffer).

Fig. 71.—Diplococcus pneumoniæ in exudate from human lung; anilin-water-fuchsin; Weichselbaum prep. (Kolle and Wassermann).

lieved it to be the real cause of pneumonia. It is the generally accepted organism of the disease, and can be isolated from nearly all cases of acute croupous pneumonia. It is found in about three-quarters of all cases of pneumonia.

Diplococcus Pneumoniæ (Fränkel and Weichselbaum, 1886).—*Synonyms.—Streptococcus Lanceolatus; Pneumococcus; Diplococcus Lanceolatus; M. of Sputum Septicemia; Fränkel's Pneumococcus.*

Origin.—Found it in the sputum of pneumonic patients. It has been found in many other serous inflammations, and also in the mouths of healthy persons.

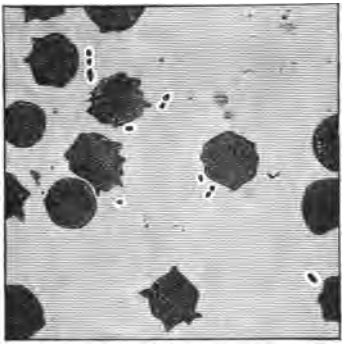


Fig. 72.—Diplococcus of pneumonia in blood of rabbit (×1000) (Fränkel and Pfeiffer).

Form.—Large, lancet-shaped cocci. Usually found in pairs, sometimes in filaments of three and four elements. In the material from the body a capsule surrounds each coccus. In the artificial cultures this is not found (Figs. 71 and 72).

Properties.—Variable in form, approaching the bacillary type. Do not liquefy gelatin. There are no spores. Non-motile.

Growth.—Best between 27° C. and 41° C., seldom below 25° C. Facultative anaërobic. The culture-media must be slightly alkaline; the growth is slow.

Colonies.—Glucose or Glycerin Agar Plates.—Growth slow, of small, round, moist colonies, separated.

Stab-cultures.—Along the needle-track small separate white granules, one above the other, like a string of beads.

Blood Bouillon.—Bouillon containing one-third blood-serum or ascitic fluid favors the growth. They grow better here than in the other media, remaining alive a longer period of time.

Blood-serum or Blood-agar.—Growth more vigorous. A good growth on blood-serum or blood-agar.

Fig. 73.—Pneumobacillus in blood (×1000) (Fränkel and Pfeiffer).

Staining.—Takes Gram's method and the other anilin stains very readily. The *capsule* stained by Hiss method (p. 61) or Welch.

Resistance.—Cultures in sugar media must be frequently transplanted, as the organism is destroyed in a few days by the acid generated. In albumin alkaline media (blood-serum, etc.) the cultures can be kept active two weeks or more. In sputum the pneumococcus may survive several days. When dried but exposed to sunlight, death occurs in a few hours.

Pathogenesis.—Rabbits and guinea-pigs, if subcutaneously injected, die in the course of a couple of days with septicemia (0.1 c.c. of a fresh bouillon culture suffices).

Autopsy shows greatly enlarged spleen and myriads of micrococci in the blood and viscera, the lungs not especially affected. If injected into the trachea, a pneumonia occurs. In man they are found in 90 per cent. of croupous pneumonia, and usually only during the existence of the rusty sputum, *i. e.*, the first stage. Found in the tissue of the inflamed lung, and in the blood in nearly all cases of lobar pneumonia.

The pneumococcus has also been found in pleuritis, peritonitis, pericarditis, meningitis, and endocarditis. It stands in some intimate relation to all infectious inflammations of the body. Their presence in healthy mouth secretion does not speak against this, it requiring some slight injury or lowered resistance to allow this ever-present germ to produce a pneumonia from an infectious disease like measles or influenza.

Toxins and antitoxins have not been separated or demonstrated. The poisons are probably endotoxins, and closely connected with the cell-body. Agglutination properties of pneumonia blood serum, if any, are very weak—1 : 50.

Immunity and Serum Therapy.—One attack produces no *immunity;* and no immune serum has been found of any value. By growing in an acid medium, the organism has been rendered less virulent.

Bacillus Pneumoniæ (Friedländer, 1882).—*Synonym.*—*Capsule Bacillus of Pfeiffer.*—Once supposed to be a cause of pneumonia. It grows readily on ordinary media; is Gram negative; in form and capsule formation it sometimes resembles the pneumococcus (Fig. 70).

Bacillus of Rhinoscleroma (Frisch, 1882).—It was found in the tissue of a rhinoscleroma, but resembles the Friedländer bacillus in nearly every respect, and as the disease rhinoscleroma is not reproduced by the inoculation of the bacillus in animals, it can be considered identical. The

growth, cultures, and properties are the same as the pneumobacillus of Friedländer.

Bacillus of Influenza (Pfeiffer, 1892).—*Origin.*—One of the smallest of the known bacilli, 1.5 μ by 0.3 μ, about one-half the size of the bacillus of mouse septicemia, and arranged in chain form. It develops upon blood-serum agar. It is aërobic, without movement (Fig. 74).

Stain.—It is best stained with diluted carbol-fuchsin, the

Fig. 74.—Bacillus influenzæ, from a gelatin culture (×1000) (Itzerott and Niemann).

contrast-stain being Löffler's methylene-blue; does not take the Gram stain.

Growth.—Upon blood-agar or glycerin-agar, over which a drop of blood has been spread, in an incubator at 37° C. at the end of twenty-four hours a very delicate growth occurs which resembles condensed moisture. Very small colonies, never larger than a pinhead, feebly resistant. Subcultures must be made every few days.

Pathogenesis.—It is found in the sputum and in the bronchial and nasal secretions and blood of influenza patients. It

has been transmitted to monkeys; other animals are not susceptible. It has never been found outside the body. Its resistance is very feeble; in water, the bacilli die in twenty-four hours, but sputa containing the germs may be ejected for days and weeks. Influenza bacilli are found accompanying bronchopneumonia, tuberculosis, meningitis, and other inflammations. The bacillus is found in healthy individuals, to a considerable extent in the nasal secretions, and it is probably spread in the fine droplets of mucus expelled in sneezing and coughing.

Koch-Weeks Bacillus (1883–87).—Cause of epidemic conjunctivitis, or "pink eye"; found in the secretion.

Form.—Very minute bacillus, resembling the influenza bacillus; non-motile. (See Fig. 84, p. 173.)

Growth.—They grow best on blood-serum agar, but very sparsely in minute transparent colonies; non-liquefying.

Stains.—With carbolfuchsin, and is often intracellular. Does not take Gram.

Pathogenesis.—Very contagious, found in 10 per cent. to 20 per cent. of all cases of conjunctivitis. Not infectious for lower animals, and not causing any other form of disease.

Bacillus of Pertussis (Whooping-cough) (Bordet-Gengou, 1906).—It has been shown that very minute bacilli resembling the influenza bacillus occur in the cilia of the cells lining the trachea and bronchi of persons affected with whooping-cough; these bacilli interfere with the normal movement of the cilia, and cause an irritation producing symptoms peculiar to the disease.

Morphology.—Very minute bacilli with rounded ends (Fig. 75).

Cultures.—On potato-blood-agar, after twenty-four hours, slight growth, sticky, grayish; subcultures made on blood-serum and veal-agar grow readily.

Staining.—Gram-negative, stain lightly with ordinary dyes.

Pathogenesis.—By inhalation inoculation young rabbits were made to develop a spasmodic cough, and the bacillus was recovered from the trachea and from bronchi in pure cultures. In the sputum of persons affected with whooping-

cough the bacillus is found in large numbers. The recent work of Mallory, Henderson, and Horner (Jour. Med. Research, March, 1913) seems to establish this organism as the real cause of pertussis.

Bacterins made from the culture have been recommended to allay the spasmodic cough.

Bacillus Melitensis (Bruce, 1887).—*Synonym.*—*Micrococcus Melitensis.*—Malta fever, also known as Mediterranean fever, occurs in the region from which it derives its

Fig. 75.—The Bordet-Gengou bacillus of whooping-cough. Twenty-four-hour-old culture upon solid media containing blood (Bordet-Gengou).

name, but has been observed in India, the Philippine Islands, and Porto Rico. Bruce cultivated an organism from the spleen and proved its specificity.

Origin.—Is found most abundantly in the spleen.

Form.—Rounded or oval, very small, coccus-like bacilli, 0.5 μ in diameter, singly, in pairs, or short chains.

Properties.—Non-motile, though flagella said to be present; grows slowly, best at body-temperature.

Gelatin.—Not liquefied; growth very slow.

Bouillon.—Turbid, with sediment.
Agar.—Pearly white growths.
Potato.—Slight invisible growth.
Stained by ordinary anilin dyes. *Gram negative.*
Glucose broth, *unfermented.*
Milk made alkaline.

The disease may be produced in monkeys by even small amounts of pure culture. In man a chronic, remittent febrile disease is produced, with sweating and arthritis. The mortality is 2 per cent. A reaction can be obtained and is diagnostic.

Agglutination—1:30 dilution of serum will give positive result, but the *complement-fixation test* considered more *certain* (*which see*).

Flies an agency for transmission.

Mode of Transmission.—Zammitt found that 50 per cent. of the goats of Malta gave the agglutination reaction to the micrococcus, and it was present in the milk in 10 per cent. Monkeys fed on the milk contracted the disease.

Preventive measures instituted in 1906 have borne out the theory that the milk of goats is the cause of Malta fever, and since the practice of importing goats from Malta has stopped, the disease has disappeared from Gibraltar. In Malta, among the troops, the fever has been greatly reduced by eliminating milk from the dietary.

CHAPTER XXIII

PYOGENIC COCCI

NEARLY all micro-organisms can produce suppuration, but in the acute abscesses occurring in the skin and lymphatics and accompanying all pus affections are found groups of micrococci so regularly that they have been designated as the pus-forming or pyogenic cocci. The two most important mem-

bers of this group are the *Staphylococcus pyogenes*, and the *Streptococcus pyogenes*, so named from the mode of division, the former being found usually in clusters or bunches, the latter in chains.

Streptococcus Pyogenes (Rosenbach): Streptococcus Erysipelatis (*Fehleisen*).—*Origin.*—Fehleisen in 1883 discovered this microbe in the lymphatics of the skin in erysipelas, and he thought it the cause of the same. Under the name Streptococcus pyogenes, Rosenbach described an identical coccus which has been found in nearly all suppurative conditions.

Fig. 76.—Streptococcus pyogenes; culture upon agar-agar two days old (Fränkel and Pfeiffer).

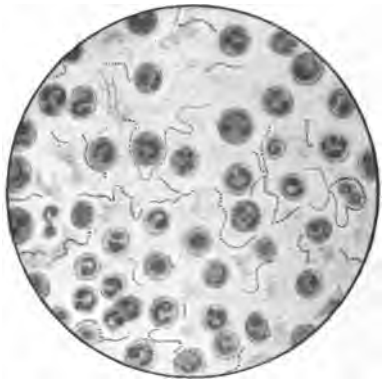


Fig. 77.—Streptococcus pyogenes (Jakob).

Form.—Small cocci singly and in chain-like groups. Spores have not been found (Fig. 77).

Properties.—They are immotile; do not liquefy gelatin.

Growth.—They grow slowly, usually on the surface, and best at higher temperatures.

Colonies.—In three days a very small grayish speck, which hardly ever becomes much larger than a pin-head; under microscope, looking yellowish, finely granular, the edges well defined.

Stab-cultures.—Along the needle-track little separated colonies, like strings of beads, which after a time become one solid white string.

Stroke-culture on Agar.—Little drops, never coalescing, having a bluish tint, very transparent.

Potato.—No apparent growth.

Bouillon.—At 37° C. clouds are formed in the bouillon, which then sink to the bottom, and long chains of cocci found in this growth.

Löffler's Blood-serum and Serum Bouillon.—Development more abundant in serum media.

Milk.—Good growth; produce lactic acid and coagulate milk.

Preservation of Cultures.—In ice-chest, the cultures may be kept alive several weeks at room temperature; they usually die out in ten days.

Staining.—Easily colored with the ordinary stains. Gram's method is also applicable.

Pathogenesis.—Inoculated subcutaneously in the ear of a rabbit, an erysipelatous condition develops in a few days, rapidly spreading from point of infection.

The micro-organism acts variously, depending upon the nature of the lesion from which it originally was obtained. Injected into the circulation, septicemia results. The more virulent the affection, the more virulent the strain.

In man, inoculations have been made to produce an effect upon carcinomatous growths, and *erysipelas* has always resulted. When it occurs upon the valves of the heart, *endocarditis* results. *Puerperal fever* is caused by the microbe infecting the endometrium, the *Streptococcus puerperalis* of Fränkel being the same germ.

In scarlatina, variola, yellow fever, cerebrospinal meningitis, and many similar diseases, the microbe has been an

almost constant attendant. It is often associated with the diphtheria bacillus in true diphtheria, and is the cause of many of the diphtheritic complications. It is associated with the influenza bacillus in acute ear suppurations; with pneumonia bacteria; with tubercle bacilli, and in such instances usually causes high fever. In osteomyelitis and mastoiditis it is usually the sole cause.

Streptococci in Milk.—In milk streptococci are often found, but it is not considered an absolute indication of udder inflammation.

Protective Sera.—An antistreptococcic serum has been used as a curative agent in puerperal fever, scarlatina, and other diseases supposed to be due to this germ. The antistreptococcic sera have been given an extensive trial in a variety of suppurative and inflammatory diseases, but the results are still under discussion.

Coley's Fluid.—A mixture of a culture of pyogenes and prodigiosus has been used as an injection, with apparent benefit, in inoperable cases of sarcoma, and is known as Coley's fluid.

Immune Bodies.—Neither antitoxic nor bactericidal bodies have been found in the blood of animals made resistant by the injection of dead or attenuated cultures.

Polyvalent (Vaccines) Bacterins.—As it is possible that there are several varieties of streptococci, and varying in pathogenic properties, bacterins made from several strains have been used as injections against suppurative processes, and with some degree of success. Autogenous bacterins are more reliable.

Distribution.—Streptococci can often be found in air, dust, on the skin, on all the mucous surfaces, pharynx, conjunctiva, tonsils.

Staphylococcus Pyogenes Aureus (Rosenbach).—*Origin.*—Found commonly in pus (80 per cent. of all suppurations), in air, water, and earth; also in sputum of healthy persons.

Form.—Micrococci in clusters like bunched grapes, hence

the name *staphylo*, which means grape. They never form chains. Spores have not been found, though the cocci are very resistant (Fig. 78).

Properties.—Immotile; liquefying gelatin. Giving rise to an orange-yellow pigment in the various cultures.

Growth.—It grows moderately fast at ordinary temperature, and can live without air, a facultative aërobin and anaërobin.

Colonies on Gelatin.—On second day small dots on the surface, containing in their center an orange-yellow spot. The

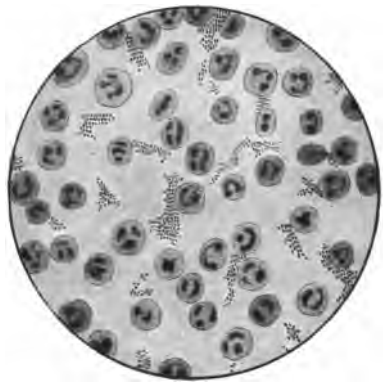


Fig. 78.—Staphylococcus pyogenes albus (Jakob).

gelatin all around the colony is liquefied; the size is never much greater than that attained the second day.

Colonies on Agar.—The pigment remains for a long time.

Stab-culture.—At first, gray growth along the track, which, after three days, has settled at the bottom of the tube in a yellow, granular mass, the gelatin being all liquid (Fig. 79).

Stroke-culture on Agar.—The pigment diffused over the surface where the growth is in moist masses.

Potato.—A thin white layer which gradually becomes yellow and gives out a doughy smell.

Staining.—Very readily colored with ordinary stains; also with Gram's method.

Pathogenesis.—When rabbits are injected with cultures of this microbe into the knee-joint or pleura, they die in a day. If injected subcutaneously, only a local action occurs, namely, abscesses.

If directly into circulation, a general phlegmonous condition arises, the capillaries become plugged with masses of cocci, infarcts occur in kidney and liver, and metastatic abscesses form in viscera and joints. Garré, by rubbing the culture on his forearm, caused carbuncles to appear.

Fig. 79.—Stab-culture. Micrococcus pyogenes aureus.

Several varieties of the pyogenic staphylococci are recognized according to their color-producing properties and slight variations of growth. Of these, the Staphylococcus pyogenes aureus is the most virulent, and is considered the type of the group. They are always present on the surface of the body, beneath the nails, in the nose and mouth, in the dust of streets, and on the floors of houses, and are found in nearly all suppurative processes, whether on the surface or internally.

Staphylococcus pyogenes albus differs from the preceding only in the absence of pigment and in its slight virulence. Welch describes a variety constantly found both on the skin and in its deeper layers, which he calls the *Staphylococcus epidermidis albus.*

Specific Therapy.—Sera have been found of no special value.

Bacterins (*Vaccines*).—Twenty-four-hour-old agar surface culture killed by heating at 60° C. is emulsified with normal saline solutions and injected for the treatment of boils, abscesses, and acne. The cultures should be autogenous, *i. e.*,

derived from the person affected, although stock vaccines have been used with some success.

The Opsonic Index.—It was proposed by Wright that the opsonic index should be obtained before treatment with vaccines, although most of the treatment is now given without such control.

Micrococcus Pyogenes Citreus (Passet).—This liquefies gelatin less rapidly than the pyogenes aureus, and forms a citron-yellow pigment instead of the orange-yellow of the aureus.

Micrococcus Cereus Albus (Passet).—Differs from the

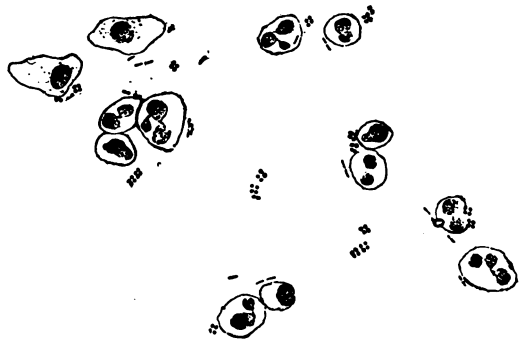


Fig. 80.—Micrococcus tetragenus in sputum (tubercle bacillus also).

pyogenes albus in the form of colony. A white, shiny growth, like drops of *wax;* hence the name, *cereus.*

Micrococcus Cereus Flavus (Passet).—A lemon-yellow colored growth after a short time, otherwise not differing from *cereus albus.*

Micrococcus Pyogenes Tenuis (Rosenbach).—*Origin.*—Found in the pus of large inclosed abscesses.

Form.—Cocci, without any especial arrangement.

Properties.—Not much studied.

Growth.—Cultivated on agar, it forms clear, thin colonies; along the needle-track an opaque streak, looking as if varnished over.

Micrococcus Tetragenus (Koch; Gaffky).—*Origin.*—Koch found this microbe in the cavity of a tuberculous lung. Gaffky, in 1883, studied its pathogenic actions and gave it the name it now bears.

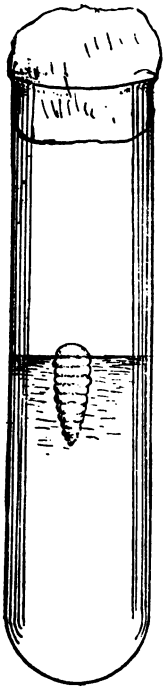


Fig. 81.—Stab-culture. Micrococcus tetragenus.

Form.—Cocci which are gathered in the tissues in groups of four, forming a square—a tetrad. (See Fig. 80.) In artificial culture sometimes found in pairs. A capsule of light, gelatinous consistence surrounds each tetrad.

Properties.—They are immobile; do not liquefy gelatin.

Growth.—They grow well on all nutrient media at ordinary temperature; are facultative aërobic. They grow slowly.

Colonies in gelatin plates. In two days, little white spots, which, when on the surface, form little elevations of a porcelain-like appearance; under low power they are seen very finely granulated.

Stab-culture.—Small, round, separated colonies along the needle-track, and on the surface a button-like elevation—a form of "nail culture." (See Fig. 81.)

Potato.—A thick, slimy layer which can be loosened in long shreds.

Staining.—Colored with the ordinary anilin stains. Gram positive.

Pathogenesis.—White mice and guinea-pigs die in a few days of septicemia when injected with the tetragenus cultures, and the micrococcus is then found in large numbers in the blood and viscera. Field-mice are immune.

In the cavities of tuberculous lungs, in the sputum of phthisical and healthy patients, it is often found, but what action it has upon man has not yet been determined.

Morax-Axenfeld Diplobacillus of Conjunctivitis.—This bacillus is found in the greater number of cases of conjunctivitis.

Form.—A short, plump bacillus, usually in pairs and chains of pairs. Non-motile (Fig. 82).

Growth.—With difficulty in blood-serum agar, it forms small pitted colonies or lacunæ; liquefies.

Staining.—Does not take Gram, but stains readily.

Non-pathogenic for lower animals.

Fig. 82.—Morax-Axenfeld diplobacillus from conjunctival exudate during course of subacute conjunctivitis (obj. B. and L., one-twelfth oil-immersion) (Boston).

Bacillus Pyocyaneus (Gessard).—*Synonyms.*—*Bacillus fluorescens* (Schröter); the bacillus of bluish-green pus.

Origin.—Found in 1882 in green pus in pyemia. Has been found in water, in bandage material, in feces and street dust, in the mouth of healthy individuals, and in all suppurating conditions, especially in middle-ear discharge.

Form.—Small slender rods with rounded ends, easily mistaken for cocci. Often in groups of four and six, without spores.

Properties.—Very motile; liquefy gelatin rapidly; a peculiar sweetish odor and a blue pigment are produced in the cultures.

Growth.—Develops readily at ordinary temperature, growing quickly and mostly on the surface; it is aërobic. *Agar plate:* In two or three days a greenish iridescence appears over the whole plate.

A bright green at first, causing fluorescence; then later a blue pigment in deeper portion.

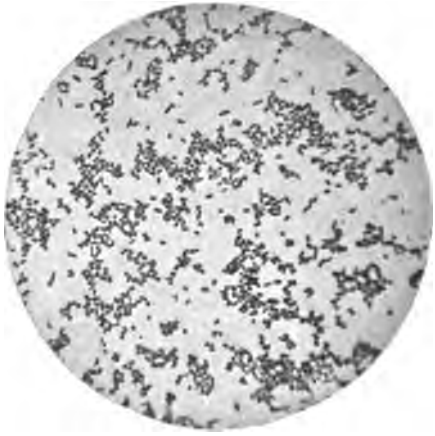


Fig. 83.—Bacillus pyocyaneus, from an agar-agar culture (× 1000) (Itzerott and Niemann).

Gelatin Stab-cultures.—Mainly in upper strata, the liquefaction funnel shaped, the growth gradually settling at the bottom, a rich green shimmer forming on the surface, and the gelatin having a deep fluorescence.

Potato.—The potato is soaked with the pigment, a deep fold of green occurring on the surface.

Indol is produced.

In ear abscesses pure cultures have been found.

Bacillus fluorescens, found in water, is considered identical

with Bacillus pyocyaneus and other fluorescent bacteria are believed to be varieties.

Crystals develop on agar cultures in a short time.

Staining.—With ordinary anilin dyes. Gram negative.

Pathogenesis.—When animals are injected with fresh cultures in the peritoneal cavities or cellular tissues, a rapidly spreading edema with general suppuration develops. The bacilli are found in the viscera and blood.

If a small quantity is injected, a local suppuration occurs,

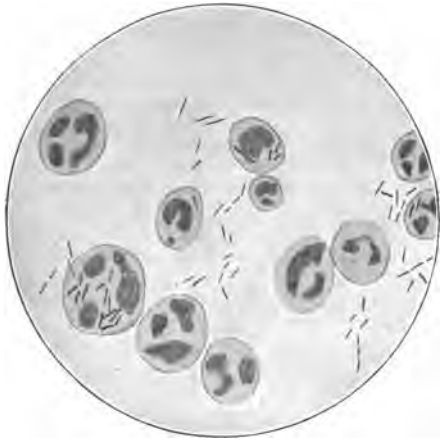


Fig. 84.—Koch-Weeks' bacillus from conjunctival exudate at third day of epidemic conjunctivitis (Boston).

and if the animal does not die, it then can withstand large quantities. It is immune.

The Pigment.—Pyocyanin.—When the pus, bandages, and dressings containing the Bacillus pyocyaneus are washed in chloroform, the pigment is dissolved and crystallizes from the chloroform in long needles. It is soluble in acidulated water, which is turned red thereby, and when neutralized, the blue color returns. It has no pathogenic action. It is an aromatic compound. The bacillus has no especial action on the wound, and is found sometimes in perspiration of healthy persons.

CHAPTER XXIV

GONOCOCCUS.—MENINGOCOCCUS

Micrococcus Gonorrhœæ (Gonococcus Neisser).—In 1879 Neisser demonstrated the presence of this germ in the secretion of specific urethritis.

Form.—Cocci, somewhat triangular in form, found nearly always in pairs, the base of one coccus facing the base of the other and giving the appearance of a Vienna roll, hence the German name, Semmel (roll), form. Four to twelve such pairs are often found together. Immotile (Fig. 86). In pus usually within the cells.

Fig. 85.—Gonococci in gonorrheal pus. Anilin methyl-violet (× 650).

Culture.—No growth on ordinary media; on blood-serum or agar smeared with blood, cultures have been obtained. The temperature must be between 33° and 37° C., and the growth occurs very slowly and sparsely.

Wertheim's medium (*q. v.*, p. 78) has given the best results.

Colonies.—Extremely delicate, translucent spots, separate, and of a slimy consistence, appearing in one to two days.

Resistance.—The cultures live only a few days at room temperature, but in the ice-chest they last longer. A temperature of 45° C. destroys the gonococci and it is but slightly resistant to the ordinary chemic antiseptics.

From the Blood.—In septicemic cases the gonococcus has been isolated from the blood direct by drawing 5 to 10 c.c. from a vein and adding it in equal parts of melted agar. The mixture is poured into Petri dishes and developed in the incubator at 37° C.

Staining.—Colored easily with all ordinary anilin stains.

Gram negative is one of its main diagnostic features.

The following method is recommended by Neisser:

The cover-glasses, with some of the urethral discharge smeared upon them, are covered with a few drops of alcoholic solution of eosin, and heated for a few minutes over the flame. The excess of the dye is removed with filter-paper, then the cover-glass placed in concentrated methylene-blue (alcoholic solution) for fifteen seconds, and rinsed in water.

The gonococci are colored dark blue, the protoplasm of the

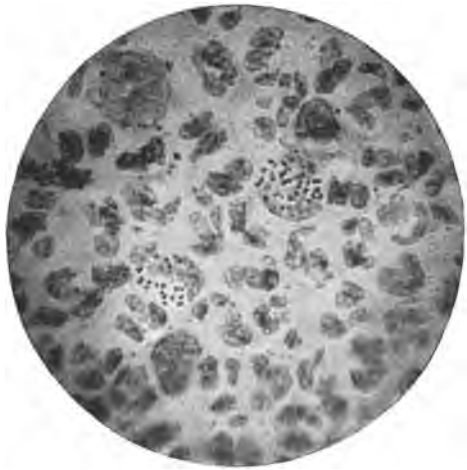


Fig. 86.—Gonococcus in urethral pus (× 1000) (Fränkel and Pfeiffer).

cell pink, and the nucleus a light blue, the gonococci lying in the protoplasm next to the nucleus (Fig. 85).

Bacterial Diagnosis.—Other bacteria are similar to the gonococci in form; they are distinguished from the gonococcus in that they are *positive* with Gram's method. The points on which the diagnosis is to be made are the *characteristic biscuit shape*, the *intracellular position of the organism*, its *failure to stain with Gram* and very *difficult* to *grow artificially* on common media.

Pathogenesis.—The attempts to infect the experiment animals with gonorrhea have so far been without success. In man, upon a healthy urethra a specific urethritis was produced with even the twentieth generation of the culture. *Gonorrheal ophthalmia* contains the cocci in great numbers, and endocarditis and gonorrheal rheumatism are said to be caused by the cocci.

The micrococci have been found long after the acute attack, when only a very slight oozing remained, and the same were found very virulent.

The specific inflammations of the generative organs of the female are due to this organism gaining entrance through the vagina. It is found chiefly in the superficial layers of the mucous membrane.

Bacterins (Vaccines).—A number of vaccines have been prepared in recent years for the treatment of gonorrhea and its complications. The bacterins are made as described under Bacterins. This method of treatment is still on trial. The best results have been obtained in gonorrheal rheumatism and epididymitis.

Toxins.—True toxins not found, but the cells contain poisons that produce suppuration and death when injected into the mice and guinea-pigs.

Allied Varieties.—A number of diplococci which resemble the gonococcus in form are found in the vaginal secretions and pus and may at times lead to a wrong diagnosis. The *meningococcus* is very similar, but is easily cultivated and is not apt to be found in the same secretions as the gonococcus.

Micrococcus citreus, albicans, and *subflavus,* described by Bumm, are all Gram positive and grow readily on gelatin and agar.

The *gonococcus* is distinguished from all these similar micrococci by the tests enumerated above.

These characteristics, taken *in toto,* form sufficient features for its ready recognition, and as it is often a serious question to decide, not so much because of the patient's health as because of his character, we should be very careful not to pronounce a

verdict until we have tested the micro-organism as above. When the germ is found which answers to the above description, the process can be called gonorrhea without a doubt.

Diplococcus Intracellularis Meningitidis (Weichselbaum).—*Synonyms.—Meningococcus; Micrococcus Meningitidis.*

Origin.—Found by Weichselbaum in epidemic cerebrospinal meningitis in 1887.

Fig. 87.—Diplococcus intracellularis meningitidis in leukocytes. Cover-glass preparation from peritoneal exudate in a guinea-pig (×2000) (Wright and Brown).

Form.—A small coccus occurring in pairs, flattened against each other, and contained within the leukocytes, resembling gonococcus. No capsule (Fig. 87).

Properties.—Ferments sugars, with acid production.

Growth.—Best on blood-agar, serum-agar, and ascitic glucose-agar at body temperature; good growth in twenty-four hours. Sheep serum better medium.

Colonies.—Circular discs, whitish, almost transparent, margins, smooth.

Stain.—With basic anilin. Gram negative. Jenner's blood-stain and Neisser stain best for spinal fluid specimens. Löffler's alkaline methylene-blue a good stain.

Resistance.—Organisms very perishable one to three days. Apparently destroyed by a self-elaborated ferment. Sunlight destroys in a few hours.

Pathogenesis.—Causes epidemic cerebrospinal fever, probably by infection through the nasopharynx; the organism is found in the spinal fluid and in other inflammatory exudates, and can be seen in fluid obtained by lumbar puncture.

Ordinary laboratory animals immune, but Flexner has succeeded in inoculating monkeys.

Agglutination.—On the fourth day; in dilution of 1 : 50 agglutination is had.

By the use of large quantities of meningococci injected into a horse agglutinins, opsonins, and specific immune bodies (amboceptor) can be produced.

Protective Serum.—Flexner has been able to obtain an antitoxin from monkey serum that has therapeutic properties in man. Such an antiserum, when injected directly into the spinal canal, has a curative action, destroying the cocci.

Bacterial Diagnosis.—By means of lumbar puncture the spinal fluid is obtained and allowed to settle. Smears made from sediment. Examined for bacteria.

Gram-positive organisms are either pneumococci, streptococci, or staphylococci.

Meningococcus is *Gram negative* and within the leukocytes, and can be readily grown on blood-serum. If such an organism is present, the disease is undoubtedly cerebrospinal meningitis.

Bacillus of Soft Chancre, Chancroid (Ducrey-Unna, 1889).—A diplobacillus which is specific has been described by Ducrey as obtained from the secretion and in the depth and margins of the chancroid. Unna's bacillus is narrower and unbroken in the center (Fig. 88).

Cultivation.—Cultivation has occurred on blood-agar, the blood being added in the proportion of one to two. *Colonies* are small, round globules.

Staining.—With borax, methylene-blue, decolorized with weak acetic acid.

Pathogenesis.—Probably a mixed infection occurs in most

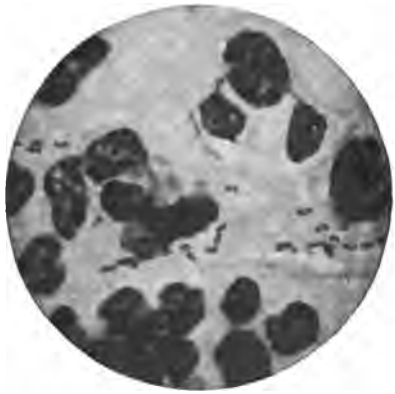


Fig. 88.—Smear of pus of chancroid of penis (× 1500) (Davis) (photomicrograph by Mr. L. S. Brown).

chancroids, especially if buboes result. The bacillus of Ducrey is not found in unopened buboes, though often contaminating the ulcerated ones.

The disease has been reproduced by inoculation of the human subject. Laboratory animals are immune.

CHAPTER XXV

ANAËROBIC BACTERIA (BACILLUS OF TETANUS; BACILLUS OF MALIGNANT EDEMA, ETC.)

SIMILAR in form and cultural requirements are a group of bacteria which are found as a result of injury or the infection of wounds. They vary greatly in the clinical symptoms produced.

Bacillus of Tetanus (Nicolaier-Kitasato).—*Origin.*—Nicolaier found this bacillus in the pus of a wound in one who had died of tetanus, describing it in 1884.

Kitasato isolated and cultivated this germ (1889).

Form.—A very slender rod.

When the spores form, a small swelling occurs at the spore end, giving the bacillus a drum-stick shape (Fig. 89).

Properties.—Not very motile, though distinctly so; liquefies gelatin slowly. The cultures give rise to a foul-smelling gas.

Growth.—Develops very slowly, best at 36° to 38° C., and only when all oxygen is excluded—an *obligatory anaërobin.* In an atmosphere of hydrogen it flourishes.

Colonies on gelatin plates in an atmosphere of hydrogen. Small colonies. After four days a thick center and radiating, wreath-like periphery, like the colonies of Bacillus subtilis. Pure cultures not easy to obtain (Fig. 90).

High Stab-culture.—The gelatin having 2 per cent. glucose added and filling the tube. Along the lower portion of the needle-track, a thorn-like growth, little needle-like points shooting out from a straight line. The whole tube becomes clouded as the gelatin liquefies, and then the growth settles at the bottom of the tube (Fig. 91).

Agar.—On agar, in the incubator, the growth is quite rapid, and at the end of forty-eight hours gas-bubbles have formed and the growth nearly reached the surface.

Bouillon.—Adding glucose to the bouillon gives a medium in which an abundant growth occurs.

Stab-agar.—Inverted fir-tree appearance.

Milk.—Acid reaction and slow coagulation.

Inoculation of animals with suspected material may be necessary as preliminary step.

Cultivation from Spores.—Kitasato, by exposing a portion of suspected material to a temperature of 80° C. for one hour,

Fig. 89.—Bacillus of tetanus with spores (× 1000) (Fränkel and Pfeiffer).

killed off all the other bacteria, but the spores of tetanus escaped and these then vegetated.

Staining.—All the ordinary stains, Gram's method also, the spores being colored in the usual way.

Pathogenesis.—A small amount of the pure culture injected under the skin of experiment animals will cause, in two to three days, death from true tetenus, the tetanic condition starting from the point of infection. At the autopsy nothing characteristic or abnormal is found, and the bacilli have dis-

appeared, except near the point of entrance. This fact is explained as follows:

Toxins.—Several toxic products have been obtained from the cultures, and they are produced in the body and give rise

Fig. 90.—Bacillus tetani: culture four days old in glucose-gelatin (Fränkel and Pfeiffer).

Fig. 91.—Six days' culture of bacillus of tetanus in gelatin (deep stab) (Fränkel and Pfeiffer).

to the morbid symptoms. These have been isolated, and when injected singly, cause some of the tetanic symptoms.

Tetanospasmin, the most important for man.

Tetanolysin.—The blood and the urine contain the toxin and are fatal to animals.

The virus enters the circulation, but does not remain in the tissues. The toxin is most virulent. It acts on the end-plates of the muscles, and then on the motor nerve-cells. The incubation period is from two to fourteen days after receipt of injury. The spores are very resistant to heat, drying, and chemicals.

Burns and injuries from firearms, cartridges, powder, and fireworks, a common cause of tetanus.

Immunity.—Kitasato, by inoculation of sterilized cultures, has caused immunity to the effects of virulent bacilli.

An *antitoxin* obtained by Tizzoni and Cattani from the serum of animals made immune by sterilized cultures is used with curative effects in cases of tetanus in man. It is a globulin, but differs from the diphtheria antitoxin. By precipitation with alcohol and drying *in vacuo* the antitoxin is obtained in a solid state. The aqueous solution is used for injection subcutaneously or subdurally through a trephine opening. Its injection into the spinal canal by lumbar puncture has also been recommended. Antitoxin is more beneficial in chronic cases than in acute.

The dried antitoxin has been spread on the wound with some curative action.

The antitetanic serum, to be effective, must be given very early and in large doses. Its greatest use is in preventing tetanus in wounds liable to be infected. From 50 c.c. to 100 c.c. of a billion-unit serum should be given in divided doses; only sera with very high protective powers should be used.

United States Government Unit for Tetanus Antitoxin.—"The immunity unit is ten times the least quantity of antitetanic serum necessary to save the life of a 350-gram guinea-pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory at Washington."

Habitat.—The bacillus is present in garden-earth, in manure, and it has been isolated even from mortar.

The earth of special districts seems to contain the bacilli in greater quantities.

Spores of tetanus may gain access to animal sera, and if not properly destroyed, may produce tetanus during the use of these products. Previous testing for the tetanus bacillus should be made in the manufacture of all animal vaccines, antitoxins, etc.

Bacillus Œdematis Maligni (Koch, 1881); Vibrion Septique (Pasteur, 1875).—*Synonym.—Bacillus Œdematis.*

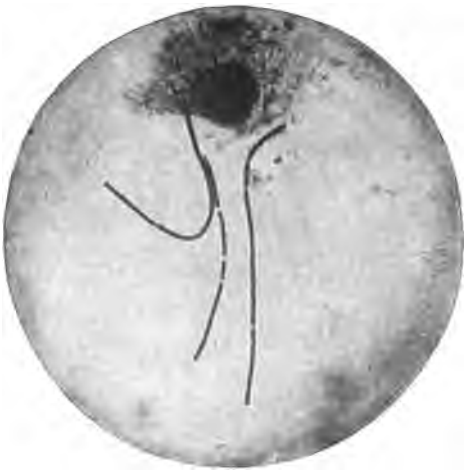


Fig. 92.—Bacillus of malignant edema, from the body-juice of a guinea-pig inoculated with garden-earth (× 1000) (Fränkel and Pfeiffer).

Origin.—In garden-earth, found also in severe wounds in man when gangrene with edema had developed. Identical with the bacillus found in *Pasteur's septicemia.*

Form.—Rods somewhat smaller than the anthrax bacillus, the ends rounded very sharply. Long threads are formed. Very large spores which cause the rods to become spindle shaped. Resembles in form and culture *B. chauvei* (Fig. 92).

Properties.—Very motile; liquefies gelatin; gas is produced in cultures but very little in the body.

Growth.—Grows rapidly, but only when the air is excluded, and best in incubator at 37° C.

Roll Cultures (After Esmarch's Method).—Small, round colonies with fluid contents, under low power, a mass of motile threads in the center, and at the edges a wreath-like border.

High Stab-culture.—With glucose gelatin, the growth at first seen in the bottom of the tube, with a general liquefaction of the gelatin; gases develop and a somewhat unpleasant odor.

Agar.—The gases develop more strongly in this medium, and the odor is more prominent.

Guinea-pig Bouillon.—In an atmosphere of hydrogen clouding of the entire culture-medium without any flocculent precipitate until third day. *Milk* coagulated. *Glucose media* marked gas fermentation.

Staining.—Are stained with the ordinary dyes, but Gram's method negative.

Pathogenesis.—When experiment animals, mice or guinea-pigs, are injected with a pure culture under the skin, they die in eight to fifteen hours, and the following picture presents itself at the autopsy: In guinea-pigs from the point of infection, spreading over a large area, an edema of the subcutaneous tissues and muscles, which are saturated with a clear red serous exudate, free from odor, and containing great quantities of bacilli.

Fig. 93.—Bacillus of malignant edema growing in glucose-gelatin (Fränkel and Pfeiffer).

The spleen is enlarged, especially in mice. The bacilli are

not found in the viscera, but are present in great numbers on the surface, *i. e.*, in the serous coverings of the different organs; though when any length of time has elapsed between the death of the animal and the examination, they can be found in the inner portions of the organs, for they grow well upon the dead body. In man they have been found in rapidly spreading gangrene following wounds.

Habitat.—They are present in the soil, in putrefactions of various kinds, and in dirty water.

Immunity.—Is produced by injection of the sterilized cultures, and also the filtered blood-serum of animals dead with the disease.

Bacillus Aërogenes Capsulatus (Welch, 1891).—*Synonym.—Bacillus Welchii; B. of Phlegmonous Emphysema* (Fränkel).

Origin.—The intestine of man and animals, soil, sewage, and water.

Form.—A thick bacillus, 3 to 6 μ in length, frequently capsulated.

Properties.—Not motile, anaërobic, forms spores chiefly in cultures on blood-serum. *Gram positive.*

Growth.—Best at 37° C.

Gelatin.—Liquefied slowly or not at all.

Bouillon.—Forms gas.

Milk.—Coagulated and becomes acid. *Under anaërobic conditions.*

Potato.—Thin, grayish-white growth with gas-production.

Forms gas in abundance in dextrose, lactose, or saccharose media.

Pathogenesis.—Is not usually pathogenic for rabbits and mice, though in guinea-pigs and birds it produces "gas phlegmons." It is sometimes found in autopsies on human subjects, producing bubbles or cavities in the viscera (Schaum-organe), but this is probably due to postmortem migration of the germ from the intestine. It has been recovered from the blood during life, however, and is the most frequent cause of *emphysematous gangrene.* In man, infection of *wounds,* through dirt, with this bacillus causes rapid emphy-

sema of the wound and a thin offensive discharge and fatal outcome. After death the bacillus develops rapidly and through the blood-vessels brings on general emphysema, with large accumulation of hydrogen gas in all the organs and subcutaneous tissue. Various foreign observers have described organisms having similar properties, and have given them such names as Bacillus perfringens, B. enteritidis sporogenes, Granulobacillus immobilis, B. saccharobutyricus,

Fig. 94.—Bacillus aërogenes capsulatus (from photograph by Professor Simon Flexner).

but they were probably dealing with the Bacillus aërogenes capsulatus.

Bacillus Enteritidis Sporogenes (Klein, 1895).—Regarded as identical with *B. aërogenes capsulatus* (*q. v.*).

Bacillus Chauvei.—*Synonyms.*—*Bacillus of Symptomatic Anthrax* (Bollinger and Feser); *Rauschbrand* (German); *Charbon symptomatique* (Arloing, Cornevin, and Thomas).

Origin.—This bacillus, described in 1879, has been isolated, and by animal inoculation shown to be the cause of the "black-leg" or "quarter-evil" disease of cattle.

Form.—Large slender rods, which swell up at one end or in the middle for the spore (Fig. 95).

Properties.—They are motile, and liquefy gelatin quite rapidly.

A rancid odor is developed in the cultures.

Cultures.—The growth occurs slowly, and only in an atmosphere of hydrogen, being anaërobic; grows best at 38° C.; under 15° C. no growth.

Glucose-gelatin.—In a few days little round colonies develop,

Fig. 95.—Bacilli of symptomatic anthrax, with spores (× 1000) (Fränkel and Pfeiffer).

which, under low power, show hairy processes around a compact center.

Stab-cultures in Full Test-tubes.—The first growth in the lower portion of the tube not very characteristic. Gases develop after a few days, and the gelatin becomes liquid.

Agar at brood temperature, in twenty-four to forty-eight hours, an abundant growth with a sour odor and abundant gas-formation.

Staining.—Ordinary methods. Gram's method is negative, but the spores can be colored by the regular double stain for spores.

Sugar Media.—Gas production.

Milk.—Rendered acid and coagulated.

Variability.—Great variation in cultures.

Toxin elaborated in fluid media fatal for rabbits when injected intravenously.

Pathogenesis.—If a small amount of the culture be injected under the skin of a guinea-pig, in twenty hours a rise of temperature, pain at the site of injection, and a few hours later death, occur. At the autopsy, the tissues are found blackened in color and soaked with a bloody, serous fluid; in the connective tissue large collections of gas, but only in the neighborhood of the point of infection. The bacilli are found in great numbers in the serum, but only appear in the viscera some time after death, when spores have developed.

The animals are usually infected through wounds on the extremities; the stalls or meadows having been soiled by the spore-containing blood of animals previously dead of the disease. "*Rauschbrand*" is the German name; "*Charbon symptomatique,*" the French, from the resemblance in its symptoms to anthrax.

Feeding experiments and infection from animal to animal negative.

Dried virus inoculation practised by the United States Government as preventive.

Immunity.—Rabbits, dogs, pigs, and fowl are immune by nature, but if the bacilli are placed in a 20 per cent. solution of lactic acid and the mixture injected, the disease develops in them. The lactic acid is supposed to destroy some of the natural resistance of the animal's cells.

Immunity is produced by the injections of these weakened cultures, and also by some of the products which have been obtained from the cultures.

CHAPTER XXVI

HEMORRHAGIC SEPTICEMIA GROUP

Bacillus of Bubonic Plague (Yersin and Kitasato, 1894).—*Synonym.*—*Bacillus Pestis.*—Bubonic plague or pest is an extremely infectious disease, more or less common in China and the East, and is believed to have its origin in man from rats and other rodents. It spreads with great rapidity,

Fig. 96.—Bacillus pestis in smear from rat's liver, showing bipolar staining (× 720) (Wherry).

especially among those living under unsanitary conditions. The "Black Death" of the fourteenth century and the plague epidemics of the seventeenth century are said to have been the same disease.

Nearly at the same time Yersin and Kitasato, working independently, discovered in the bubonic swellings and blood

of affected persons a distinctive bacillus which has conformed to all the conditions necessary to make it the cause of the disease.

Origin.—In the tissues and all the body-fluids and secretions of affected individuals.

Form.—Short, thick rods with an indistinct capsule and rounded ends. Growing in chains in fluid media (Fig. 96).

Properties.—Immotile. Stains readily. No spores. Cultivated best in oxygen, but is facultative anaërobic. Stains stronger at the ends, producing bipolar appearance. Gelatin not liquefied. *Easily destroyed by sunlight and drying.* Very resistant to cold.

Growth.—Best at 30° C.; aërobic.

Gelatin.—At 22° C., in twenty-four hours, white, point-like colonies on the plates, with broad and flat surface, turning gray and then brown. Milk not curdled; slightly acid.

Stab.—Snow-white, spreading out on the surface to the edge, and fluorescent.

Bouillon.—Granular precipitate, with clear fluid above. When covered with oil and kept at rest, filaments hang down from surface like stalactites.

Agar and Blood-serum.—Glass-like colonies like drops of dew at first, then growing larger with iridescent edges.

Potato.—At 37° C. small white mass. Slow growth.

No gas formation in glucose media.

Staining readily with all basic dyes. Gram negative. Capsule found in agar growths.

Pathogenesis.—After subcutaneous injection in rats death follows in forty to sixty hours, with symptoms of severe toxemia and convulsions. The point of infection shows a local edema and inflammation of the lymphatics. All the organs congested and surrounded by a bloody exudate. The characteristic bacilli in all the tissues and secretions. Nearly all the domestic animals are susceptible. Mosquitos and pigeons, however, are immune—flies are not; fleas are a very important element in the transmission, and the rat-flea may communicate the disease to the rat from man or from the rat to

man. Infected ground squirrels are supposed to be a factor in spreading the disease. Animals protected from the flea may live near infected animals without danger. Direct infection by dust or other material seldom occurs. The sputum of patients having the pneumonic type is highly infectious. Close personal contact with the infected is a means of transmission. The main point of entrance is the skin. Fifty per cent. of wild rats immune and not easily affected.

Products.—A toxin has been obtained and immunity has been effected; the serum of immune animals has protective properties. The serum likewise shows *agglutinating* powers, and gives similar reactions to typhoid and cholera sera.

Habitat.—Not found in water, but most likely spreads from the soil in damp and darkened areas. Rats become affected first, and then, through fleas, affect man and other animals. In man three forms of the disease are recognized according to the mode of infection and course of the disease—viz., bubonic, pulmonic, septicemic.

Vaccines.—The vaccines of Haffkine and Terni and Bandi have been used extensively, and with some good results.

Antitoxins.—The antitoxins of Yersin and of Lustig have been used, but without much result. Closely identified with *Bacillus pestis* is the group known as the hemorrhagic septicemia bacteria

Bacteria of Hemorrhagic Septicemia (Hueppe, 1886).—Under this heading Hueppe has gathered a number of bacteria very similar to the bacillus of chicken cholera, differing from it and each other but very little. They have been described by various observers and found in different diseases.

The bacteria of this group color themselves strongly at the *poles*, giving rise to the dumb-bell shape (Fig. 97). They do not take the *Gram stain;* they are without spores, and do not liquefy gelatin.

They have been divided into three groups, *Bacillus avisepticus*, as it appears in fowls; *Bacillus bovisepticus*, as it attacks cattle; *Bacillus suisepticus*, as it attacks swine. The prominent members of each group are: Bacillus of

chicken cholera of Pasteur, bacillus of swine plague, and bacillus of cattle-plague or pleuropneumonia.

Bacillus of Chicken Cholera (Perroncito, Pasteur, 1878).—*Synonyms.—Micrococcus cholera gallinarum; Microbe en huit; avicidus bacillus; bacillus of fowl septicemia.*

Origin.—In 1879 Perroncito observed this coccus-like bacillus in diseases of chickens, and Pasteur, in 1880, isolated and reproduced the disease with the bacillus in question.

Form.—At first it was thought to be a micrococcus, but it has been found to be a short rod, about twice as long as it is

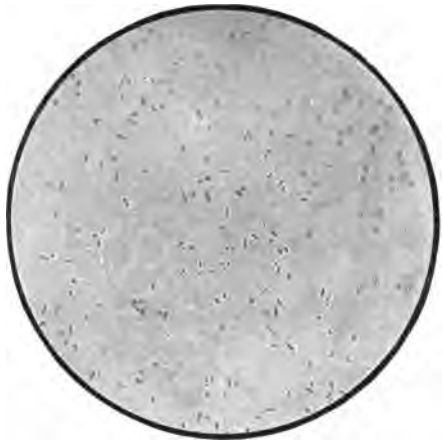


Fig. 97.—Bacillus of swine-plague (from photograph by E. A. de Schweinitz).

broad, the ends slightly rounded. The center is very slightly influenced by the anilin colors, the poles easily, so that in stained specimens the bacillus looks like a dumb-bell or a figure-of-8 (Microbe en huit).

Properties.—Does not possess self-movement; does not liquefy gelatin; no spores.

Growth.—Occurs at ordinary temperature, requiring oxygen for development. It grows very slowly.

Gelatin Plates.—In the course of three days little round, white colonies, which seldom increase in size, having a rough border and very finely granulated.

Stab-cultures.—A very delicate gray line along the needle-track, which does not become much larger.

Agar Stroke Culture.—A moist, grayish-colored skin, more appreciable at brood-heat.

Potato.—At 37° C., after several days, a very thin, transparent growth.

Sugar Broth.—Acid fermentation, no gas.

Indol is formed.

Staining.—Methylene-blue gives the best picture. Gram's method is not applicable. As the bacillus is easily decolorized, anilin-oil is used for dehydrating tissue sections, instead of alcohol.

Fig. 98.—Chicken cholera in blood (× 1000) (Fränkel and Pfeiffer).

Pathogenesis.—Feeding the fowls with the bacilli or injecting them under the skin will cause death in from twelve to twenty-four hours, the symptoms preceding death being those of a severe septicemia.

The bacillus is then found in the blood and viscera and the intestinal discharges, the intestines presenting a hemorrhagic inflammation.

Guinea-pigs and sheep are immune. Mice and rabbits are affected in the same manner as the fowls.

Immunity.—Pasteur, by injecting different-aged cultures into fowls, produced in them only a local inflammation, and they were then immune. But as the strength of these cultures could not be estimated, many fowls died and the healthy ones were endangered from the intestinal excretions, which is the chief manner of infection naturally, the feces becoming mixed with the food.

Bacillus of Erysipelas of Swine (Löffler, Schütz).—*Synonyms.*—*Schweinerotlaufbacillus* (German); *Rouget du Porc* (French).

Origin.—Found in the spleen of an erysipelatous swine by Löffler in 1885.

Form.—One of the smallest forms of bacilli known; very thin, seldom longer than 1 μ, looking at first like little needle-like crystals. Spores have not been found.

Properties.—They are motile; do not liquefy gelatin.

Growth at ordinary temperature very slowly, and the less oxygen, the better the growth.

Gelatin Plate.—On third day little silver-gray specks, seen best with a dark background, coalescing after a while, producing a clouding of the entire plate.

Stab-cultures.—In a few days a very light, silvery-like clouding, which gradually involves the entire gelatin; held up against a dark object, it comes plainly into view.

Staining.—All ordinary dyes and Gram's method also.

Tissue sections stained by Gram's method show the bacilli in the cells, capillaries, and arterioles in great numbers.

Pathogenesis.—Swine, mice, rabbits, and pigeons are susceptible; guinea-pigs and chickens, immune.

When swine are infected through food or by injection, a torpidity develops with diarrhea and fever, and on the belly and breast red spots occur which coalesce, but do not give rise to any pain or swelling. The animal dies from exhaustion in twenty-four to forty-eight hours. In mice the lids are glued together with pus.

At the autopsy the liver, spleen, and glands are enlarged and congested, little hemorrhages occurring in the intestinal mucous membrane and that of the stomach.

Bacilli are found in the blood and in all the viscera.

One attack, if withstood, protects against succeeding ones.

Immunity.—Has also been attained by injecting vaccines of two separate strengths.

Bacillus Murisepticus (Koch); Mouse Septicemia.—*Origin.*—Found in the body of a mouse which had died from injection of putrid blood, and described by Koch in 1878.

Form.—Differs in no particular from the bacillus of swine erysipelas, excepting that it is a very little shorter, making it the *smallest* known bacillus. Spores have been found, the cultures exactly similar to those of swine erysipelas.

The pathologic actions are also similar. Field-mice are immune, whereas for house and white mice the bacillus is fatal in two to three days.

Micrococcus of Mal de Pis (Nocard).—Gangrenous mastitis of sheep.

Origin.—In the milk and serum of a sheep sick with the "*mal de pis.*"

Fig. 99.—Bacillus of mouse septicemia, from the blood of a mouse (× 1000) (Fränkel and Pfeiffer).

Form.—Very small cocci, seldom in chains.

Properties.—Immotile; liquefying gelatin.

Growth.—Growth occurs best between 20° and 37° C., is very rapid, and irrespective of oxygen.

Plates of Gelatin.—White round colonies, some on the sur-

face and some in the deeper strata, with low *power*, appearing brown, surrounded by a transparent areola.

Stab-culture.—Very profuse along the needle-track, in the form of a cone after two days, the colonies having gathered at the apex.

Potato.—A dirty gray, not very abundant, layer, somewhat viscid.

Staining.—With ordinary methods; also Gram's method.

Pathogenesis.—If a pure culture is injected into the mammary gland of sheep, a "*mal de pis*" is produced which causes the death of the animal in twenty-four to forty-eight hours. The breast is found edematous, likewise the thighs and perineum; the mammæ very much enlarged, and at the nipples a blue-violet coloration. The spleen is small and black; other animals are less susceptible. In rabbits abscesses at the point of infection, but no general affection.

CHAPTER XXVII

PROTOZOA

PROTOZOA are unicellular animal organisms, minute as bacteria, and differing from bacteria in the methods of reproduction. Their structure and functions are more complex, although the borderland is ill defined. A nucleus is usually present.

Divisions.—There are four grand divisions of protozoa: (1) *Sarcodina*, containing 5500 species; (2) *mastigophora*, containing 500 species; (3) *infusoria*, containing 700 species; (4) *sporozoa*, containing 300 species.

Sarcodina are chiefly marine forms, with processes changeable in shape. *Examples:* Ameba, foramnifera, entameba, parasitic for man.

Mastigophora have undulating flagella and are known as flagellates; to this division the trypanosomata belong. *Example:* Trypanosoma.

Infusoria have fine ciliary processes or numerous delicate flagella. *Example:* Balantidium.

Sporozoa have no motile organs, and are reproduced by spores. To this division belong the coccidia of malaria and the organisms discovered by Mallory in scarlatina. *Examples:* Plasmodium, coccidium.

Life-cycle.—The complete cycle of reproduction has been observed in only one of the pathogenic protozoa, namely, the protozoa of malaria.

Methods of Cultivation.—Novy, Clegg and others have obtained pure cultures of protozoa by the use of blood-agar and animal tissue, or by cultivation with bacteria, on which the ameba and other protozoa live.

Entamœba Histolytica (Shaudinn, 1903).—Amœba Dysenteriæ.—Found in the intestinal ulcers, feces, and secondary liver abscesses in certain cases of dysentery. Kartulis, in 1886, definitely established the cause, although amebæ were noted in feces by Lambl in 1860. A non-pathogenic form, Amœba coli, also occurs. The Amœba dysenteriæ is a unicellular animal organism, measuring 25 to 35 μ in diameter, though larger and smaller forms occur. A nucleus and a nucleolus are present; the protoplasm of the cell-body is vacuolated, and often contains red blood-cells and bacteria. In fresh, warm stools active ameboid motion may be observed. The non-pathogenic form is smaller and never contains red blood-cells.

Examination for Amebæ.—From the slimy part of the fresh feces a loopful is taken and diluted with salt solution and examined with moderate power on a warm stage. Look for contracting vacuole and motion.

Staining with hematoxylin eosin or eosin methylene-blue after the film on a glass slide or cover-glass has been fixed in hot alcohol or methyl alcohol.

Cultures.—On nutrient agar a loopful of feces is spread and examined from day to day, transplanting the young amœbæ with their accompanying bacteria.

Pathogenesis.—Inoculation experiments with monkeys and

dogs produce dysentery and liver abscess. In man, 50 per cent. of human beings harbor non-pathogenic amebæ, but the pathogenic variety is found mainly in tropical countries, where it produces serious lesions and often occurs in widespread epidemics.

Source.—It is supposed to come from poor water supplies. Amebic dysentery differs from the bacillary form in that no severe toxic symptoms are present and the amebic disease is more chronic. The Shiga bacillus, B. dysenteriæ, is found in the bacillary form of dysentery.

Life Cycle of the Malarial Sporozoa.—According to its situation, the parasite exhibits two distinct phases of existence: in the human blood it passes through an *asexual* reproductive cycle, known as *schizogony*, while in the body of the mosquito it undergoes an entirely different series of *sexually* reproductive changes, called *sporogony*.

1. *The Asexual Cycle in Man.*—An infected mosquito conveys the parasites into the blood of man as minute hyaline bodies which enter the blood-cells. At first they are small, round, colorless bodies, exhibiting more or less active ameboid motion in the fresh blood. Sometimes, particularly in the estivo-autumnal form, a ring shape is assumed. Their size gradually increases and pigment-granules appear, while in stained specimens a nucleus containing chromatin granules is visible. As the parasite approaches maturity the chromatin becomes scattered, and finally the protoplasm or mother-cell, known as *sporocyte*, divides into six to twenty spores, daughter-cells or *merozoites*, each containing a portion of the chromatin. The number of spores formed and their arrangement before segmentation takes place differ in the three varieties and will be noted below. The spores burst through the envelop of the red corpuscle and become free in the blood, but speedily enter fresh corpuscles and pass through the same series of changes. The febrile stage is synchronous with sporulation and liberation of the young forms.

Certain of the parasites do not, however, go on to segmentation, but after reaching maturity, remain quiescent and form

the so-called *gametes* or sexual types. In the tertian and quartan varieties these are not very different from the mature

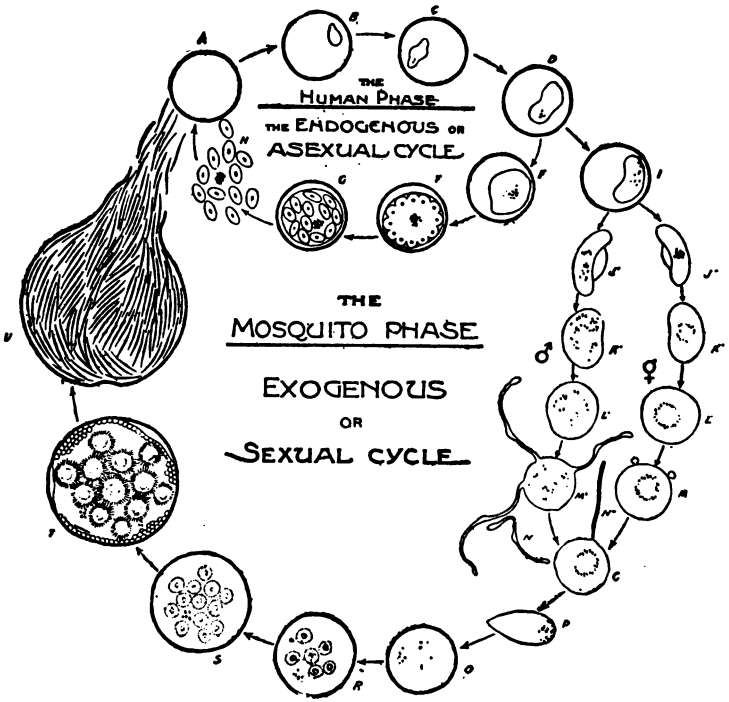


Fig. 100.—Schema showing the human and mosquito cycles of the malarial parasite: A, Normal red cell; B, C, D, E, red cells containing amebulas or myxopods; F, G, H, sporocytes; J′, K′, L′, M′, microgametocytes or male gametes; J″, K″, L″, M″, O, macrogametocytes, or female gametes; N′, M′, microgametes; P, traveling vermicule; Q, young zygote; R, S, zygotomeres; T, blastophore; U, mature zygote (modified from Blanchard's diagram illustrating life-cycle of Coccidium schubergi) (Rees, in "Practitioner," March, 1901).

organisms, but the estivo-autumnal gametes are crescentic in shape and very characteristic.

2. *The Sexual Cycle in the Mosquito.*—The common mosquito is known as Culex and does not harbor the malarial parasite. The anopheles species, spotted wings, is the true host; only the females are bloodsuckers and responsible for the spread of the disease. They take the infected blood containing the male element and which represents the male fertilizing element (*microgametes*). These become detached, and, entering a female gamete (*macrogamete*), a true sexual fertilizing process takes place. In the alimentary canal of the mosquito these fertilized cells penetrate the stomach-walls and form cysts (oöcysts) filled with a large number of filiform spores (sporozoïtes), which are extruded into the body cavity of the insect, and some of which reach the salivary glands, whence they are ejected when the mosquito bites. This cycle of development takes seven or eight days.

Three Forms of Malarial Protozoa.—1. *Plasmodium Vivax, or The Tertian Form.*—The adult forms are large, not very refractile, and their outline is somewhat indistinct. There is an abundance of fine pigment-granules, and the ameboid motion is vigorous. Segmenting forms divide into fifteen to twenty merozoites; the sexual forms or gametes are large. The red cell containing the organism is swollen and pale. Sporulation and, therefore, the malarial paroxysm occur every forty-eight hours.

2. *Plasmodium Malariæ, the Quartan Form.*—The organism is smaller, is more refractile, and its outline is more distinct. The pigment is coarse and situated at the periphery of the organism, while the protoplasmic motion is sluggish. Segmentation forms only six to twelve spores, and has the regular "daisy-head" appearance; the gametes are small. The red cells become dark in color, and the cycle requires seventy-two hours.

3. *Plasmodium Falciparum, or Malignant Tertian, or Estivo-autumnal Form.*—The adult forms are found mainly in the spleen and other viscera, and do not very often occur in the peripheral blood; their outline is sharp, and they are highly refractile. The pigment is scanty and fine; the motion is

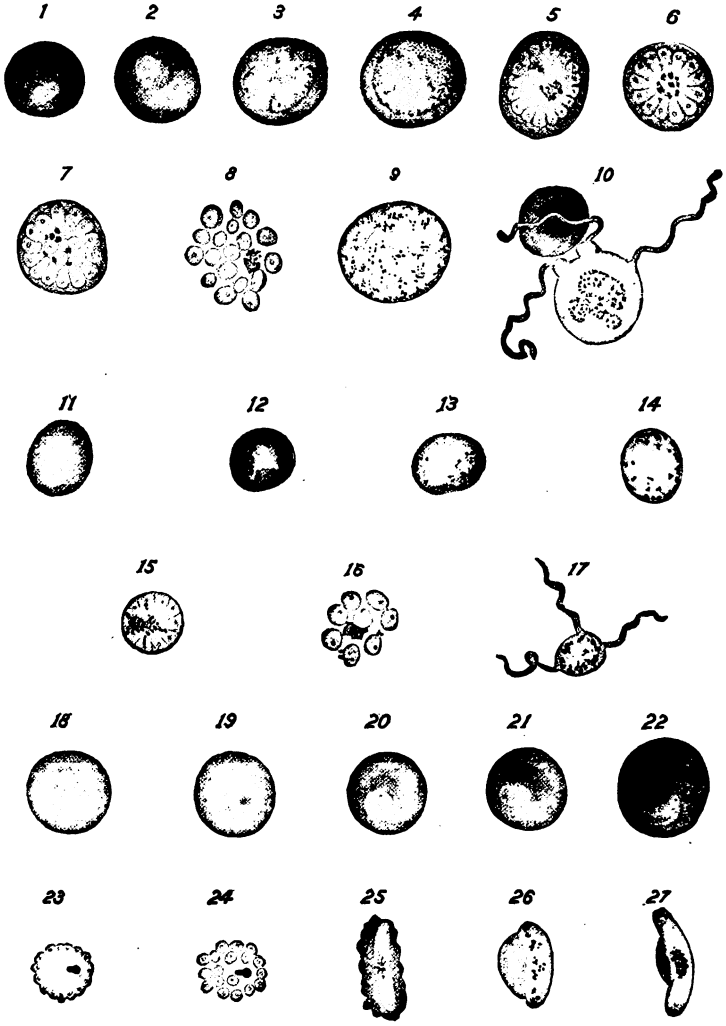


Fig. 101.

active. A variable number of merozoïtes is formed—usually six to twelve. The gametes are characteristic, being crescentic in shape and very resistant to quinin. The red cell becomes shriveled and yellowish. The cycle usually takes forty-eight hours, though it is somewhat variable.

Mixed infections with the different organisms or with two or more broods of the same organism may occur, so that quotidian and irregular paroxysms may be produced.

Transmission.—Malaria is spread by means of a mosquito, the *anopheles*, in whose body the protozoön undergoes its highest development. Man is the intermediate host; the mosquito, the true host.

Methods of Examination for Malarial Organisms.—1. *Fresh preparations* are made by placing a small drop of blood on a slide and a cover-glass over it, so that only a thin film is formed. A ring of vaselin is smeared over the edges of the cover-glass to prevent evaporation. This is the best method for studying flagellation and fertilization, but is less satisfactory for routine clinical work than—

2. *Stained Smears.*—These are made by spreading a drop of blood in a thin film over one slide with the edge of another, drying in the air, and staining. Many stains have been devised for the malarial organism, but Jenner's or Wright's is sufficient for ordinary use:

(1) *Jenner's Stain.*—This is excellent for routine work, as no preparatory fixation is required. The smears are dropped into this stain for one to three minutes, without previous fixation, and at once rinsed in distilled water. The malarial parasites are stained blue, the cell-bodies a reddish brown.

Fig. 101.—Various forms of malarial parasites (Thayer and Hewetson): 1–10 inclusive, tertian organisms; 11–17 inclusive, quartan organisms; 18–27 inclusive, estivo-autumnal organisms.

1, Young hyaline form; 2, hyaline form with beginning pigmentation; 3, pigmented form; 4, full-grown pigmented form; 5, 6, 7, 8, segmenting forms; 9, mature pigmented form; 10, flagellate form.

11, Young hyaline form; 12, 13, pigmented forms; 14, fully developed form; 15, 16, segmenting forms; 17, flagellate form.

18, 19, 20, Ring-like and cross-like hyaline forms; 21, 22, pigmented forms; 23, 24, segmenting forms; 25, 26, 27, crescents.

(2) *Wright's Chromatin Stain.*—This is the best of the chromatin stains. For its preparation, which is quite complicated, see Wright, *Journal of Medical Research*, vol. vii, 1902. It can be purchased already made. It is used as follows:

1. The stain is poured over the film and allowed to remain for one minute to secure fixation.

2. Add distilled water drop by drop until a metallic scum is formed on the surface. The staining now takes place and requires two to three minutes. Wash in distilled water until

Fig. 102.—Pure culture of trypanosomes of mosquitos—Crithidia fasciculata. Multiplication roset showing large and small cells. Nine-day culture (Gen. 1 × 1500) (Novy, MacNeal, and Torrey).

a pinkish tint appears in the thin portions of the smear. The body of the malarial parasite is stained blue, and its chromatin a lilac to red color. The red cells are orange-pink.

If possible, examinations for malarial organisms should always be made before quinin is administered.

Trypanosomata.—Trypanosomes are flagellate protozoa found in the blood of various animals, and causing a number of diseases, such as surra, dourine, and nagana, affecting horses and cattle, especially in tropical countries, and causing

the sleeping sickness of Africa, which is very fatal for human beings. About 60 species have been described, and 10 diseases are believed to be due to this form of organism.

Morphology.—A fusiform mass, containing at one end a flagellum (Fig. 103).

In the living state these protozoa are very motile. In the stained specimen chromatin granules are found and two or more nuclei. From the smaller nucleus arises the undulatory membrane, which passes into the flagellum and assists in the wave-like motion.

Fig. 103.—Pure culture of trypanosomes of mosquitos—Crithidia fasciculata. Part of roset of elongated crithidia with flagella directed centrally (Gen. 39 × 1500) (Novy, MacNeal, and Torrey).

In the body fluids division occurs, first of the nucleus and then of the protoplasm.

Cultivation.—Novy and MacNeal have succeeded in cultivating these protozoa on blood-agar, and multiplication goes on rapidly, so that *rosettes* are formed with the flagella arranged around a common center. (See Figs. 102, 103, 104.)

Trypanosoma Lewisi (Kent, 1878).—Found in rats by Lewis; not fatal to them, though often equaling the red corpuscles in number. It was one of the first of this group to

be described. The infection continues for two months without producing any illness, and the animal is then immune.

Injection of infected rat blood into healthy rat causes the latter to become infected.

The injection of serum from an immune rat will prevent the disease in normal rats.

Cultivated best at 20° C. and is very resistant to cold. The rat is probably infected by the bite of a flea or louse. (See Fig. 105.)

Fig. 104.—Pure culture of trypanosomes of mosquitos—Crithidia fasciculata. Elongated crithidia from same preparation as preceding (Novy, MacNeal, and Torrey).

Trypanosoma Brucei (Plimmer and Bradford, 1894) causes *nagana*, or *tsetse-fly disease*, a disease affecting horses, cattle, and dogs in certain regions of South Africa. The trypanosome of Bruce is less motile than that of Lewis. It has been cultivated at 25° C., and is less resistant to cold. All laboratory animals subject to infection. The rat dies in ten days.

In the natural infection Bruce discovered that the tsetse-fly transmitted the disease, but that it did so by first biting some animal whose blood contained the trypanosome. The

blood of infected animals contains the organism, and can, if injected, produce the disease without the agency of the fly. So far the tsetse-fly alone is responsible for the spread of the infection.

Sleeping Sickness.—Trypanosoma Ugandense Gambiense (Dutton, 1904).—(*T. Castellani, T. Hominis, T. Neprevi.*)—Sleeping sickness, or human trypanosomiasis, is a disease peculiar to some parts of Africa. It is accompanied by periods of fever, anemia, and, finally, a lethargy deepening

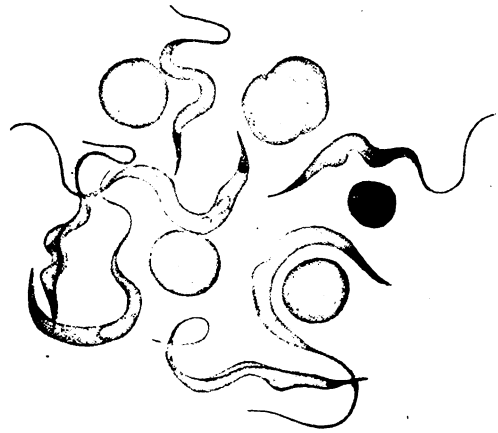


Fig. 105.—Trypanosome from blood of gray rat; stained with a 2 per cent. aqueous solution of methylene-blue (Boston).

into coma and death. The disease may be rapid, and it may last with recurrences for many years. Trypanosomes identical with those found in nagana disease have been found in the blood of infected persons, and described by various observers, and given different names.

Monkeys, when inoculated with cerebrospinal fluid from affected persons, develop a similar disease, and the parasites are found in the blood. So far the organism has not been cultivated.

A blood-sucking fly, known as the *Glossina palpalis*, is considered the means of infection. The fly is closely related to the *Glossina morsitans*, or tsetse fly. The sleeping sickness in man is most likely the same thing as the nagana of cattle.

Methods of Examinations.—From Blood.—A patient search may fail to detect the organisms—a large amount of blood, 10 c.c., obtained by venesection—is centrifuged and the white cells examined in hanging drop or stained smear.

Cerebrospinal fluid will at times give results.

Animal Inoculation.—The blood of suspected person injected into monkeys or rats and the resulting infection studied by above methods.

Staining.—The organism is best stained by Giemsa stain or the Romanowsky method.

Trypanosoma Evansi (Steel, 1880).—Pathogenic for all animals.

Discovered by Evans in the blood of horses suffering from surra, a disease prevalent in India and the Philippine Islands. The disease resembles nagana.

T. equiperdum and *T. Rougetii* are names given to similar organisms found in dourine, a disease affecting horses in southern France and Spain. Trypanosomes are found in fish, oysters, birds, and frogs, and many varieties have been described.

Herpetomonas (Leishman, 1903) (*Leishman-Donovan Bodies*).—A disease called variously kala-azar, dum-dum fever, tropical splenomegaly, is considered to be due to an organism somewhat related to the trypanosomes.

Smears are stained after fixation by the Wright or Romanowsky stains. *Cultivation* has succeeded on blood-media made acid with citric acid.

The bedbug is considered instrumental in transmitting the organism.

Piroplasma Bovis (P. Bigeminum) (T. H. Smith, 1893).—*Origin.*—In the blood of animals suffering from Texas cattle-fever.

Form.—A pear-shaped protozoön, found in pairs in the red

cells of the blood, the smaller ends of pear in opposition; coarse ameboid movement.

Transmission.—An insect or tick (Boöphilus bovis) becomes infected, and by its bite infects other animals.

Other similar sporozoa have been found in animal diseases and in man in Rocky mountain fever. The *P. hominis* has been described, but not definitely determined.

Rabies or Hydrophobia.—Negri Bodies (Negri, 1903).—*Origin.*—Found in the nervous system of animals dying of rabies (hydrophobia).

Form.—Round and oval, hyaline bodies, with a sharp outline and containing a nucleolus. The plasma is slightly granular. They are regarded as protozoa.

Staining.—A smear from brain tissue is made on a coverglass and fixed in methyl-alcohol for five minutes; then stained by Giemsa; stain for half-hour to three hours.

All mammals susceptible; man chiefly from bite of dog. Only a small percentage of persons bitten by rabid dog become infected—16 per cent.

The virus resides in the saliva, and also in the central nervous system. The Pasteur preventive is an accepted fact, and depends for its power on a form of active immunization. The virus used is obtained from dried spinal cord of infected rabbits, gradually increasing the virulence, older cords first used and then cords exposed to drying for lesser time.

CHAPTER XXVIII

THE MICRO-ORGANISM OF SYPHILIS AND ALLIED ORGANISMS

Spirochæta Pallida (Schaudinn, 1905).—*Spironema Pallidum; Treponema Pallidum.*—Found in hereditary syphilis in all organs, in chancre, and lymphatic glands, and in secondary lesions, *mucous patches*, in the internal organs,

and likewise in the tertiary lesions, the very latest being the brain, and cerebrospinal fluid in cases of general paralysis, and establishing the identity of this disease with cerebral syphilis.

Form.—A minute, spiral-shaped organism, with 6 to 20 curves, ends tapering. Actively motile in fresh specimen (Fig. 106), intracellular, and affecting glandular epithelium.

Staining.—The organism requires special staining, and a number of complicated methods have been introduced by different investigators. The Giemsa stain is said to give the best results. (See Staining Fluids, p. 47.)

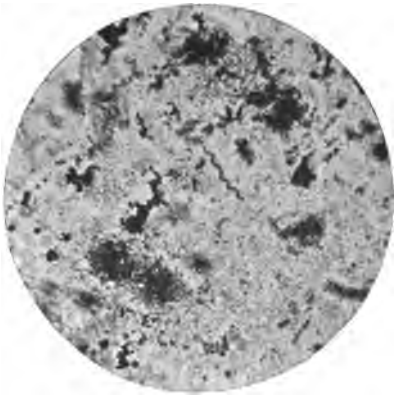


Fig. 106.—Spirochæta pallida. Microphotograph made by Dr. R. E. Lavenson from a specimen prepared by H. Fox (Stengel).

The slide is fixed, dried in air, hardened in absolute alcohol twenty-five minutes, stained with dilute stain (1 drop to 1 c.c. of water) for ten minutes, washed in water, and mounted.

In tissues the organism can be shown by fixing with silver nitrate after the manner of Ramon y Cajal. The tissue is—(1) Hardened in formalin for twenty-four hours (the sections should be thin); (2) washed in water for one hour; (3) alcohol, twenty-four hours; (4) $1\frac{1}{2}$ per cent. silver nitrate solution in incubator at 37° C., three days; (5) washed in water twenty minutes; (6) placed in mixture of pyrogallic acid, 4 parts; formalin, 5 parts; distilled water, to make 100 parts, and kept in dark bottle for forty-eight hours; (7) washed in water and alcohol and then embedded in paraffin and sectioned. Spirochætæ black, tissues, pale yellow. Or counterstain of fuchsin can be employed.

The India Ink Method.—A drop of fluid from a lesion is mixed with a drop of *India ink* upon a clean glass slide and allowed to dry. Examine with oil-immersion lens. The spirilla appear dark in a mass of carbon particles. By using dark ground illumination, the organism appears brightly refractive.

Culture Methods.—*Noguchi*, by using a serum water (1 part sheep or horse serum, 3 parts water, and adding a piece of sterile rabbit's kidney or testicle), under strict anaërobic conditions at 35° C. succeeded in cultivating the organism direct from lesions in man. After several transfers the organism will grow on agar containing the bit of tissue.

Inoculation Experiments.—Pure cultures inoculated into rabbits and monkeys produce lesions resembling the primary sores, and the blood of such animals gives a *Wassermann reaction.* Cutaneous inoculation on eyebrows and genitals of material from primary and secondary lesions produces results in from fifteen to fifty days.

Wassermann Reaction.—In 1906 Wassermann, Neisser, and Bruck described a method of making the diagnosis of syphilis by demonstrating in the blood and spinal fluid of a patient complement-binding substances not present in normal blood.

Technic.—The following reagents are employed: (1) Syphilitic antigen; (2) serum to be tested; (3) fresh guinea-pig serum; (4) washed sheep corpuscles and antisheep amboceptor.

The antigen is an alcoholic extract of liver from a congenital syphilitic, and is prepared by extracting the ground-up liver with five volumes of absolute alcohol for ten days and then filtering.

Complement is normal guinea-pig serum.

Antisheep amboceptor is obtained by injecting into a rabbit 2, 4, 6, 8, and 12 c.c. of washed sheep corpuscles on the first, tenth, nineteenth, twenty-eighth, and thirty-seventh days respectively. Nine days after the last injection the animal is bled to death from the carotid and the blood collected in

sterile test-tubes. After clotting has taken place the clear serum is removed. This is the *amboceptor serum.*

Washed sheep corpuscles are obtained by centrifuging defibrinated sheep blood, pipeting off the serum, replacing it with normal salt solution, shaking, and again centrifuging. This is repeated three times.

Patient's serum obtained from blood from the patient's arm is *heated* thirty minutes at 56° C. to destroy complement.

Titration or Testing of Reagents.—Titrate amboceptor. One c.c. of a 5 per cent. suspension of washed sheep corpuscles in salt solution and 0.1 c.c. of fresh guinea-pig serum are added to a series of test-tubes. The amboceptor serum is then added so that each tube receives more than the preceding one. Salt solution is added to make 5 c.c. and the tubes incubated for two hours at 37° C. with occasional shaking. That tube in which complete hemolysis has taken place in just two hours contains $\frac{1}{2}$ unit of amboceptor.

Titration of Complement.—Into each of a series of tubes place 1 c.c. of the corpuscle suspension and $\frac{1}{2}$ unit of amboceptor. Next add 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2 c.c. of fresh guinea-pig serum respectively and incubate for two hours, shaking occasionally. Those tubes which show complete hemolysis in just two hours contain 1 unit of complement.

Titration of Antigen.—Two-tenths c.c. of serum, previously heated to 56° C. for a half-hour, from a known, untreated case of secondary syphilis, and 1 unit of complement are added to each of a series of test-tubes. Antigen is now added, so that each tube contains more than the preceding one, and salt solution added and brought to 3 c.c. The mixture is incubated for one hour at 37° C., at the end of which time 2 units of amboceptor and 1 c.c. of corpuscle suspension are added and the tubes returned to the incubator. After a short period the tube containing the smallest amount of antigen will show complete hemolysis. As the dose of antigen is increased the amount of hemolysis is decreased until a point is reached at which no hemolysis takes place even after twenty-four hours. The first tube in the series

which shows no hemolysis after twenty-four hours contains 1 unit of antigen provided twice that amount will not prevent hemolysis when no serum is added.

Having found out the exact amount of guinea-pig serum (complement) necessary to unite with hemolytic amboceptor (rabbit serum) in order to hemolyze blood-corpuscles, this amount is mixed with syphilitic antigen plus the suspected syphilitic serum amboceptor, and incubated for one hour at 37° C. *If the amboceptor is syphilitic, it will combine with the antigen and guinea-pig complement.* To find out if the *complement has been bound*, the hemolytic amboceptor and its antigen sheep corpuscles are added to the mixture, and *if no hemolysis takes place, the complement is fixed* and the *patient's serum contains the syphilitic antibodies or amboceptors.*

To Set Up Test.—Nine tubes needed for Wassermann reaction and control. Into each tube 1 c.c. diluted *complement* guinea-pig serum. Into tubes 1, 2, and 9, 0.2 c.c. of patient's serum. Into tubes 3 and 4, control, syphilitic serum 0.2 c.c.; in 5 and 6, normal serum as control, 0.2 c.c.; antigen extract, 1 unit placed in 1, 3, 5, and 7.

To each tube is now added sufficient normal salt solution to make 3 c.c. Tubes gently shaken and placed in incubator at 37° C. one hour. At end of the hour to each tube is added 1 unit of suspension sheep corpuscles, and to all but No. 9 2 units of standard amboceptor, in 1 c.c. saline.

The tubes again placed in incubator for one hour, readings taken, and then placed in ice-box twenty-four hours, when final results noted. If Wassermann positive—

No. 1. No hemolysis.
No. 2. Complete hemolysis.
No. 3. No hemolysis.
No. 4. Complete.
No. 5. Complete.
No. 6. Complete.
No. 7. Complete.
No. 8. Complete.
No. 9. No hemolysis.

WASSERMANN SCHEME

No.	Complementary Serum	Patient's Serum	Known Syphilitic Serum	Normal Serum	Antigenic Units		Sheep Corpuscles	Amboceptor		+ Result
1	1 c.c.	0.2 c.c.	..	..	1	Incubate one hour.	1	1	Incubate one hour. Place on ice twenty-four hours.	No hemolysis.
2	1 c.c.	0.2 c.c.	..	..	..		1	1		+ Complete hemolysis.
3	1 c.c.	..	0.2 c.c.	..	1		1	1		No hemolysis.
4	1 c.c.	..	0.2 c.c.	..	..		1	1		+ Complete hemolysis.
5	1 c.c.	..	..	0.2 c.c.	1		1	1		Complete hemolysis.
6	1 c.c.	..	..	0.2 c.c.	..		1	1		Complete hemolysis.
7	1 c.c.	..	..	..	1		1	1		Complete hemolysis.
8	1 c.c.	..	..	..	..		1	1		Complete hemolysis.
9	1 c.c.	0.2 c.c.	..	..	..		1	..		As a rule, no hemolysis.

Noguchi modification of the Wassermann reaction consists in using *human corpuscles* and *antihuman amboceptor*, and, as *antigen, acetone insoluble lipoids.*

Antigen.—Extract a finely ground ox-heart with 10 volumes of absolute alcohol at 37° C. for several days; filter and evaporate the extract (using an electric fan and not heat) almost to dryness. Extract the residue with ether; decant, evaporate the ether, and redissolve in the smallest quantity of pure water-free ether. To this ethereal solution add 5 volumes of water-free acetone. A precipitate forms which is the antigen. The precipitate is dissolved in purest methyl-alcohol in the proportion of 3 per cent. For use, 1 c.c. of this alcoholic solution is mixed with 9 c.c. of salt solution.

Titration of Antigen.—(1) *Hemolytic Action.*—A tube con-

taining 0.4 c.c. of the antigen emulsion, 0.1 c.c. of 10 per cent. suspension of corpuscles, and 0.6 c.c. of salt solution should show no hemolysis after two hours at 37° C.

(2) *Anticomplementary Bodies.*—A tube containing 0.4 c.c. of antigen, 0.1 c.c. of a 40 per cent. dilution of complement, 2 units of amboceptor, and 0.6 c.c. of salt solution is incubated for one hour and 0.1 c.c. of 10 per cent. corpuscle suspension added. In two hours there should be complete hemolysis.

(3) *Antigenic Properties.*—After incubating for one hour a tube containing 0.02 c.c. antigen, 0.02 c.c. of a known syphilitic serum, 0.1 c.c. of a 40 per cent. dilution of complement, 2 units of amboceptor, and 0.8 c.c. of salt solution, 0.1 c.c. of a 10 per cent. corpuscle suspension is added, and the tube returned to the incubater. At the end of two hours there should be no hemolysis.

(4) Amboceptor and complement are titrated the same as in the Wassermann reaction, except that a 1 per cent. suspension of human corpuscles and 0.02 c.c. of complement and antihuman amboceptor are used.

NOGUCHI SCHEME

	No.	Patient's Serum	Known Syphilitic Serum	Normal Serum	Washed human corpuscle, 1 c.c.	Complement serum, 0.1 c.c. to each tube.	Antigen	Incubate one hour.	Amboceptor, 2 units to each tube.	Incubate two hours.	After Ten Hours
Patients	1	1	..	..			1				No hemolysis.
	2	1	..	..			..				Complete hemolysis.
Positive control	3	..	1	..			1				No hemolysis.
	4	..	1	..			..				Complete hemolysis.
Negative control	5	..	..	1			1				Partial, later complete.
	6	..	..	1			..				Complete hemolysis.

Explanation of Noguchi Modified.—Requires six tubes:

In 1 and 2, one drop serum to be tested; in 3 and 4, one drop known syphilitic serum; in 5 and 6, one drop normal serum.

To each tube add 1 c.c. 1 per cent. suspension washed human blood-corpuscles, and 0.1 c.c. 40 per cent. fresh guinea-pig serum (complement).

Into 1, 3, and 5, one drop antigen solution.

Incubate at 37° C. one hour, then add 2 units antihuman amboceptor to each tube. Incubate two hours and read reaction every hour for next ten hours, keeping tubes at room temperature.

Tubes 2, 4, 6, complete hemolysis.

Tube 5, complete hemolysis.

Tube 3, no hemolysis.

Tube 1, no hemolysis.

Results of Wassermann Test.—Eighty per cent. of primary cases give a positive result, but a negative reaction in this stage does not mean much, as nearly 20 per cent. of cases are negative.

95 per cent. of secondary cases give a positive reaction.

85 per cent. of tertian positive.

90 per cent. congenital forms strongly positive.

100 per cent. general paresis positive.

50 per cent. locomotor ataxia positive.

65 per cent. latent tertiary forms.

Luetin Reaction (Noguchi).—An emulsion of a pure culture of the spirochetes of syphilis heated to 60° C. for one hour, and made sterile, is called *luetin.*

When applied subcutaneously by means of a fine needle, an erythema lasting forty-eight hours results in normal persons, but in persons affected with tertiary, latent, and congenital syphilis after forty-eight hours a small induration or papule appears, which at times becomes vesicular and pustular, increasing in redness and turning bluish red in three or four days. It is an adjunct to other tests for syphilis.

Yaws.—*Spirochetes* similar and possibly identical with those of syphilis have been found in this tropical disease.

Spirillum of Relapsing Fever (Obermeier, 1873).—*Synonym.—Spirochæta Obermeieri.*

The definite classification of this organism has not been made. Some regard it now as a protozoön, and one of a group in which numerous other spirilla belong.

Origin.—Found in the blood of recurrent fever patients, described in 1873.

Form.—Long, wavy threads (16 to 40 μ long), a true spirillum; flagella are present (Fig. 107).

Fig. 107.—Spirochæta Obermeieri from human blood (Kolle and Wassermann).

Properties.—Very motile. *Has not been cultivated.*

Staining.—Ordinary anilin stains. Bismarck-brown best for tissue sections.

Pathogenesis.—Found in the organs and blood of recurrent fever. Man and monkeys inoculated with blood from one suffering from this disease become attacked with the fever, and in their blood the spirillum is again found. It is found in the blood only in the relapses (during the fever). After the attack the spirilla gather in the spleen and gradually die

there. It has been found in the brain, spleen, liver, and kidneys. In the secretions it has not been discovered.

Agglutinating substances have been developed. Immunity has been produced in rats, and the serum has antitoxic properties.

Transmission.—The bedbug retains the spirillum in its blood and is considered an important factor in spreading the disease.

African Tick Fever.—A spirochæte similar to that of relapsing fever has been observed in ticks, which conveyed a disease to monkeys similar to the above fever.

CHAPTER XXIX

FILTERABLE ORGANISMS

Filterable or Ultra-microscopic Organisms.—There are many widely distributed infectious diseases that have all the characteristics of germ or bacterial diseases, but so far the organism has not been found. It has been suggested that the bacteria are so small that they pass through the ordinary germ-filters and are beyond the powers of the microscope. By aid of the *ultra-microscope* twice the magnification of the usual oil-immersion lens can be obtained, and it is hoped that the cause of some of these diseases will thereby be ascertained. The instrument is still imperfect, though even so it has opened up a new field of research.

Such diseases as measles, foot and mouth disease of cattle, typhus fever, small-pox, scarlatina, and infantile poliomyelitis (epidemic infantile paralysis) are assumed to be due to these bacteria.

Small-pox and Vaccinia.—The exciting agent of small-pox is still unknown, but numerous bacteria and protozoön-like bodies have been described and given etiologic signifi-

cance by various authors. There is some evidence in favor of Funck's belief that vaccinia is caused by a protozoön, the *Sporidium vaccinale*. Animals inoculated with this organism developed both vaccinia and variola. It is possible that the organism causing small-pox is a filterable one, and beyond the present methods of research.

Yellow Fever.—For some years it was thought that a bacillus, called *Bacillus icteroides* by Sanarelli, was the cause of yellow fever. The earlier work of Sternberg was disproved when it was shown that his bacillus, *Bacillus X*, was identical with the colon group, and Reed and Carroll found that Sanarelli's germ was an allied organism.

It is now known that a special species of mosquito, *Stegomyia fasciata*, conveys the infection and acts as a culture-medium for some unknown microörganism, possibly a protozoön, which must undergo certain changes to become virulent.

Only by the bite of a mosquito infected with the blood of a yellow-fever patient or by direct inoculation of such blood can yellow fever be transmitted.

The experiments made so far show that the germ is destroyed by a temperature of 55° C. for ten minutes. It can pass through a Berkefeld filter, and is, therefore, extremely minute, ultra-microscopic, but no one has as yet been able to find any distinctive organism in the blood.

Measles.—Recent experiments (Anderson and Goldberger) demonstrated the virus in the nasal and mouth secretions, and this secretion, collected forty-eight hours before eruption, when inoculated into monkeys reproduced measles in them. The infection was not possible forty-eight hours after the eruption nor from the desquamation.

Typhus fever, or Brill's disease, has a virus which is non-filterable and which resides in the plasma of the blood. Monkeys can be inoculated with the disease. Transmitted by lice.

Acute Poliomyelitis.—The virus is contained in brain and spinal cord and also in the mucous membrane of the nose, in the salivary glands, and cerebrospinal fluid; it is

very little resistant to heat. Monkeys inoculated through the nose or directly into the brain. Immunity is produced and an immune serum as preventive is obtained. The stable-fly is supposed to act as a carrier of infection.

CHAPTER XXX

YEASTS AND MOLDS

In works on bacteria these true fungi, *yeasts* and *molds*, are usually considered. They are so closely related to bacteria, and so often contaminate the culture-media, and are so similar in many respects, that a description is almost a necessity.

But there are several thousand varieties, and we cannot attempt to describe even all the more important ones. A description of a few of the more common kinds must suffice.

Blastomycetes (**budding fungi**) or **yeasts** increase through budding; the spores are attached to the mother-cell like a tuber on a potato (Fig. 108).

Yeasts are the cause of alcoholic fermentation in the saccharoses, and hence called *saccharomycetes.*

Saccharomyces Cerevisiæ (**Torula Cerevisiæ**).—This is the ordinary beer-yeast.

Form.—Round and oval cells; a thin membrane inclosing a granular mass, in which usually can be seen three or four irregular-shaped spores. When these become full grown, they pass through the cell-wall and form a *daughter-cell.* Sometimes long chains are produced by the attached daughter-cells.

Growth.—They can be cultivated as bacteria are in bouillon, but grow best in beer.

Yeasts are very resistant: cultures have been obtained from material twelve years old and dry as a bone.

There are several varieties of beer-yeasts, each one giving a

characteristic taste to the beer. Brewers, by paying special attention to the nutrient media, cultivate yeasts which give to their beers individual flavors.

Mixed yeast gives rise to a poor quality of beer.

Saccharomyces Rosaceus; S. Niger; S. Albicans.—These yeasts are found in the air; and instead of producing alcoholic fermentation, they give rise to a pigment in the culture-media. They grow upon gelatin, which they do not liquefy.

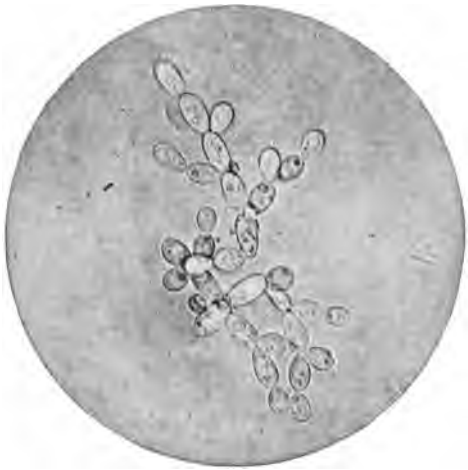


Fig. 108.—Yeast-cells (× 500) (Fränkel and Pfeiffer).

Saccharomyces Mycoderma.—This yeast forms a mold-like growth, or skin, on the surface of fermented liquids, but does not cause any fermentation itself. It forms the common "mold" on wine, preserves, and "sauer-kraut."

Oïdium.—A form which seems to be the bridge between the yeast and the molds is the oïdium. Sometimes it resembles the yeasts, sometimes the molds, and often both forms are found in the same culture. Several are pathogenic for man.

Oïdium Lactis.—*Origin.*—In sour milk and butter.

Form.—The branches or hyphæ break up into short, rod-like spores. No sporangium, as in molds.

Growth.—In milk it appears as a white mold.

Artificially cultured on gelatin plates, or milk-gelatin plates, it forms satin-like, star-shaped colonies, which slowly liquefy. Under the microscope the form of the fungus is well seen.

Agar Stroke Culture.—The little stars, very nicely seen at first; then the culture becomes covered with them, causing a smeared layer to appear over the whole surface, with a sour odor.

Properties.—The milk is not changed in any special way. It is not pathogenic for man or animals. It is found when the milk begins to sour.

Oïdium Albicans (Soor; Thrush Fungus, Langenbeck, 1839).—*Origin.*—Mucous membrane of the mouth, especially of infants.

Form.—Taken from the surface of the culture, a form like yeasts; but in the deeper layers, mycelia with hyphæ occur.

Growth.—Not liquefying; snow-white colonies on gelatin plates.

Stab-culture.—Radiating yellow or white processes spring from the line made by the needle, those near the surface having oval ends.

Potatoes.—The yeast form develops as thick white colonies.

Bread-mash.—Snow-white veil over the surface.

Pathogenesis.—In man the parasitic thrush, or "white mouth," is caused by this fungus. In the white patches the spores and filaments of this microbe can be found. Rabbits receiving an intravenous injection perish in twenty-four to forty-eight hours, the viscera being filled with mycelia.

Pathogenic Yeasts.—A number of workers have interested themselves in experiments with yeasts in their relation to disease; and under the name of *blastomycetes*, Sanfelice has grouped yeasts that produce tumors resembling epitheliomata; and he has tried to prove that the so-called animal parasites found in malignant growths, and variously known

as coccidia and sporozoa, are yeasts. These are, however, protozoa.

Blastomycetic Dermatitis or Oïdiomycosis.—A skin disease described, in 1894, by Gilchrist, and since then by other writers, is due to a fungus which resembles yeast, and which has been called a blastomyces; but Ophüls and Ricketts term it an oïdium, and the former calls the parasite *Oïdium coccidioides.*

On Löffler's blood-serum and agar a growth occurs in from three to seven days, small white colonies made up of branching, *mold-like* forms. On potato the growth is more rapid and shows the yeast forms.

The disease is slow in process,—ten to twelve years,—leaving much deformity. When generalized, it is fatal.

Form.—The fungus increases by budding, but in culture-media it may resemble a mold or oïdium.

Pathogenesis.—Small abscesses form in wart-like lesions, which extend over large areas of the skin, becoming later on systemic and invading lungs and kidneys; abscesses and nodules form in these organs.

Hyphomycetes (True Molds).—Flügge has made five distinct divisions of molds. It will, however, serve our purpose to classify those to be described under three headings: *Penicillium, Mucor,* and *Aspergillus.*

Penicillium Glaucum.—*Origin.*—The most widely distributed of all molds, found wherever molds can exist.

Molds frequently contaminate the cultures by bacteria and culture-media.

Form.—From the mycelium, hyphæ spring which divide into basidia (branches), from which tiny filaments arise (sterigmata), arranged like a brush or tuft. On each sterigma a little bead or conidium forms, which is the spore. In this particular fungus the spores in mass appear green.

Growth.—It develops only at ordinary temperatures, forming thick, grayish-green molds on bread-mash. At first these appear white, but as soon as the spores form, the green predominates. Gelatin is liquefied by it.

Mucor Mucedo.—Next to the Penicillium glaucum, this is the most common mold. Found in horse-dung, in nuts and apples, in bread and potatoes, as a white mold.

Form.—The mycelium sends out several branches, on one of which a pointed stem is formed which enlarges to form a globular head, a spore-bulb, or *sporangium.* The spore-bulb is partitioned off into cells in which large oval spores lie. When the spores are ripe, a cap forms around the bulb, the

Fig. 109.—Penicillium glaucum (× 500) (Fränkel and Pfeiffer).

walls break down, and the wind scatters the spores, leaving the cap or "*columella*" behind. The rounded sporangium is usually black.

Growth.—Takes place at higher temperatures on acid media. It is not pathogenic.

Achorion Schönleinii. Trichophyton Tonsurans. Microsporon Furfur.—These three forms are similar to each other in nearly every particular, and resemble in some respects the Oïdium lactis, in other ways, the mucors. The

first one, *Achorion Schönleinii*, was discovered by Schönlein in 1839, in *favus*, and is now known as the direct cause of this skin disease.

Origin.—Found in the scaly crusts of favus (Fig. 110).

Form.—Similar to Oïdium lactis.

Growth.—Is very sparse. Agar, at body temperature, two types—waxy, yellowish mass, and downy, white-plush-like covering.

In milk it is destroyed.

Pathogenesis.—Causes favus in man, also in animals.

Trichophyton Tonsurans ("Ring-worm").—Found, in 1854, by Bazin, in tinea.

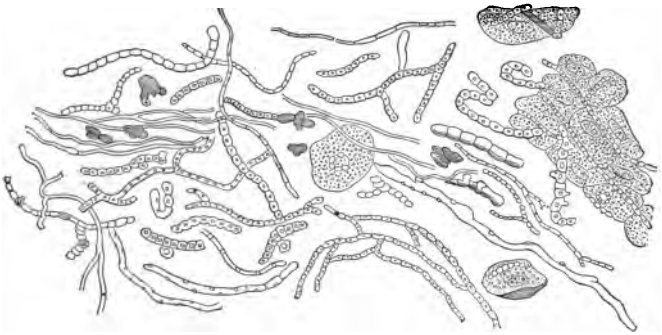


Fig. 110.—Achorion Schönleinii (after Kaposi).

Form.—Similar to the achorion or favus fungus.

Growth.—Somewhat more rapid than the favus, and the gelatin quickly liquefied. Old cultures are of an orange-yellow color. Colonies have a star-shaped form.

On agar and potato the organism can be cultivated by first treating the infected hairs and scales with potassium hydroxid (dilute solution); this liberates the spores and dissolves some of the bacteria which usually contaminates the culture. Some of the colonies are crateriform.

Pathogenesis.—Herpes tonsurans and the various tineæ are produced by this fungus.

Microsporon Furfur.—Found in tinea or pityriasis versicolor, almost identical with the above; forms dry yellow spots, usually on the chest, in persons suffering from wasting diseases.

Aspergillus Glaucus.—The aspergillus is a common mold contaminating bacterial cultures.

Origin.—In saccharine fruits.

Form.—The hypha has formed upon its further end a bulb, from which pear-shaped sterigmata arise and bear upon their ends the conidia or spores.

Fig. 111.—Aspergillus fumigatus (× 500) (Fränkel and Pfeiffer).

Growth.—Best upon fruit-juices. *Non-pathogenic.* The mold is green. *Aspergillus flavus* has the tufts and spores of a yellow color.

Aspergillus Fumigatus.—Is pathogenic for rabbits when injected into them. At the autopsy their viscera are found filled with the mold.

Examination of Yeasts and Molds.—Yeasts and molds are best examined in the unstained condition. A small portion of the colony rubbed up with a mixture of alcohol and a

few drops of liquor ammonia; of this, a little is brought upon the glass slide, covered with a drop of glycerin, and the cover-glass pressed upon it. If the preparation is to be saved, the cover-glass is secured by ringing around the edges with varnish or cement. *Yeasts* take *methylene-blue* stain very well.

Cladothrices and Streptothrices.—The streptothrix and cladothrix groups are classed with the higher bacteria, but their exact status is still undetermined. They may be con-

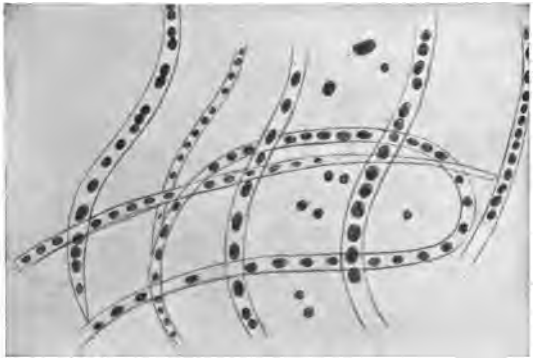


Fig. 112.—Cladothrix dichomata from well-water (one-twelfth oil-immersion. Fuchsin stain) (author's specimen).

sidered as representing transition forms from the bacteria to the lower fungi.

Crenothrix Kühniana (Rabenhorst).—Long filaments joined at one end; little rod-like bodies form in the filaments, and these break up into spores.

Zoöglea are also formed by means of spores, and these can become so thick as to plug up pipes and carriers of water. They are not injurious to health.

Cladothrix Dichotoma (Cohn).—Very common in dirty waters. The filaments branch out at acute angles, otherwise resembling the crenothrix; accumulations of ocher-colored

slime, consisting of filaments of this organism, are found in springs and streams. (See Fig. 112.)

Leptothrix Buccalis.—In the mouth, long filaments or threads resembling bacteria are commonly found. At one end are seen numerous cocci-like bodies, which some regard as spores. A variety of this, or a nearly allied organism, is the most frequent cause of noma or gangrenous stomatitis.

With iodin the leptothrix is colored yellow. At one time it was considered the cause of "tartar" on the teeth, and often it fills the crypts of the tonsils, forming there small masses which are difficult to remove. Miller distinguishes three varieties—Leptothrix buccalis innominata, maxima, and gigantea.

Beggiatoa Alba (Vancher).—The most common of this species. The distinction between this and the preceding species lies in the presence of sulphur granules contained in the structure, and hence they are often found where sulphur or sulphids exist; but where the remains of organic life are decomposing they can also be found.

Several large spirilla and vibrios live in bog and rain-water, but our space does not suffice to describe them. For the Bacteriologic Examination of Water see p. 325.

Streptothrix or Cladothrix Actinomyces (Ray-fungus).—Actinomycosis is a disease caused in man and cattle by an organism which is commonly found in grain, particularly barley. It is probable that several varieties of the parasite can produce the characteristic lesions. It has been discovered in all countries and in various organs of the body, although its place of election is about the lower jaw, where it tends to form hard, ulcerating abscesses, affecting other organs secondarily.

Form.—In the granular masses of an abscess cylindric filaments are matted together, and radiating outward from this zone are club-shaped branches, as the petals of an aster. (See Fig. 113.) In the center of the granule are numerous cocci-like bodies, and some of the ovoid or club-shaped hyphæ lie detached from the clusters. Through cultivation

it is found that the ovules give rise to filaments, and they then form the ovules again.

Cultivation.—At 38° C. on glycerin-agar in a period of one to two weeks pointed scales about the size of a millet-seed, center dry and prominent, margins hyaline, composed only of filaments, short and long, massed together, but no clubbed forms.

The *clubs* have been considered as spore organs; by

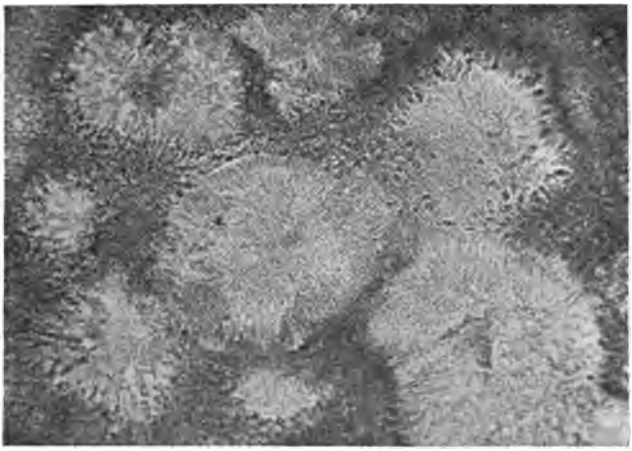


Fig. 113.—Actinomyces granule crushed beneath a cover-glass, showing radial striations in the hyaline masses. Preparation not stained; low magnifying power (Wright and Brown).

others, they are thought to be encapsulated or thickened filaments.

Pathogenesis.—When a portion of the growth obtained in eggs is injected into the abdominal cavity of a rabbit, actinomycotic processes develop upon the peritoneum.

It usually gains access to the living body through a wound in the gum or some caries of the teeth. A new growth is formed, ulceration being first set up.

The new tissue, composed of round-cells, then undergoes

softening, purulent collections form, and the normal structure is destroyed.

The usual seat is in the maxillary bones, but the fungus has been found in the lungs, tonsils, intestines, and various other organs in man and cattle.

Examination.—Well seen in the unstained condition. From the pus or scraping a small portion is taken and squeezed upon the glass slide; if calcareous matter is present, a drop of nitric acid will dissolve this.

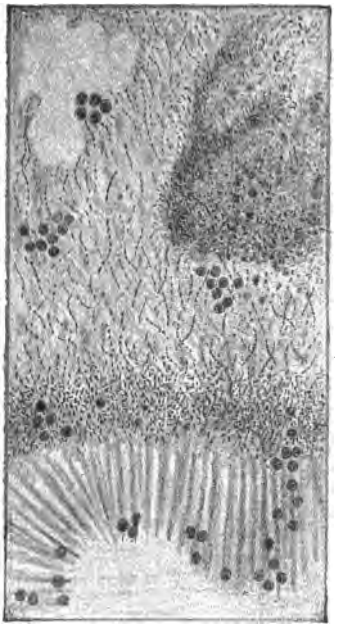


Fig. 114.—Streptothrix Maduræ in a section of diseased tissue (Vincent).

Glycerin will preserve the preparation.

Staining. — Cover-glass specimens stained best by Gram's method. Tissue sections should be stained as follows:

Ziehl's carbol-fuchsin, ten minutes. Rinse in water.

Concentrated alcoholic solution of picric acid, five minutes. Rinse in water.

Alcohol, 50 per cent., fifteen minutes. Alcohol absolute, clove-oil, balsam.

The rays stained red, the tissue yellow.

Streptothrix Maduræ (Vincent).—*Origin.*—Found in the disease known as Madura foot, or mycetoma, an ulceration affecting the feet, especially of individuals living in the tropics. Two varieties, the *pale* and the *black*, have been described.

Form.—Branched filaments resembling the actinomyces streptothrix. In the mycelia spores are seen (Fig. 114).

Cultivation.—In liquid media containing vegetable infusions growth occurs best. Temperature of 37° C. most suited. The colonies near the surface become colored red.

Agar.—Glazed colonies, at first colorless, then rose-colored, about the size of a pea, with the central part umbilicated and pale. Gradually the rose color fades.

Acid Potato.—A slow and meager growth.

Pathogenesis.—Only local reaction has been caused by inoculation in animals. In man the disease usually follows a slight injury and attacks the leg or foot, slowly forming a nodular growth, which in the course of months or a year begins to soften and ulcerate, and with the seropus are discharged numerous little granules, some black, some pink, containing mycelia. The limb becomes much deformed, the tissue vascularized, and the degenerated area filled with the streptothrix filaments.

Staining.—The organism itself stained with ordinary stains. Gram's method for the tissue.

Nocardia (Streptothrix) Farcinica (Nocard); Bovine Farcin du Bœuf.—*Origin.*—A disease affecting cattle, and giving rise to tubercle-like lesions in the lungs, liver, and spleen. Common in France.

Form.—Small interwoven mass of threads arranged in tufts found in the centers of the tubercles.

Culture.—At body-temperature in various media.

Bouillon.—Colorless masses, irregular in size and shape.

Agar and Gelatin.—Small, rounded, opaque colonies, thicker at the periphery.

Potato.—Rapid growth of pale-yellow, dry scales, consisting of many spores.

Pathogenesis.—Pure cultures introduced into the peritoneum of guinea-pigs give rise in nine to twenty days to tubercle-like lesions. Subcutaneous injections cause abscesses with secondary involvement of the lymphatics, ending in recovery. Dogs, horses, and rabbits are immune.

Staining.—Wright's double stain for tissues; also Gram's.

Plant Diseases due to Bacteria.—There are a great

variety of blights, rots, and new-growths, such as galls attacking plants, which are seemingly due to bacteria. About 30 varieties have so far been more or less accurately described, but only a few of the organisms have been definitely associated with the disease. The *pear blight* is due to *Bacillus amylovorus*. Crown gall, which affects a great many plants and trees, is supposed to be due to *Bacterium tumefaciens;* the black rot of cabbage to a pseudomonas. There is much left to be done to place this part of bacteriology on a par with that devoted to animals and man.

CHAPTER XXXI

EXAMINATION OF AIR, SOIL, AND WATER

Air.—Many germs are constantly found in the atmosphere about us. Bacteria unaided do not rise into the air and fly about; they usually become mixed with small particles of dirt or dust and are moved with the wind. The more dust, the more bacteria, and, therefore, the air in summer contains a greater number than the air in winter, and all the other differences can be attributed to the greater or less quantity of dust and velocity of the wind.

By the use of balloons, living bacteria have been found at an altitude of 4000 meters.

Methods of Examination.—The simplest method is to expose a Petri dish with gelatin or agar in a dust-laden atmosphere or in the place to be examined. In the course of twenty-four to forty-eight hours colonies will form wherever a germ has fallen. But this method will not give any accurate results in regard to the number of bacteria in a given space; for such purposes somewhat more complicated methods are used, so that a definite amount of air can come in contact with the nutrient medium at a certain regulated rate of speed.

This form of analysis, however, has not yielded any very practical results, and is not much resorted to.

Hesse's Method.—Hesse's method requires an apparatus called an *aëroscope*, which, by means of siphoning bottles (*aspirator*), sucks air through a cylinder lined with gelatin, and by regulating the rate of flow an approximate idea of the number of bacteria per liter of air can be obtained. A less complicated method is known as *Petri's method.*

Fig. 115.—Petri's sand-filter for air-examination (McFarland).

Fig. 116.—Sedgwick's expanded tube for air-examination (McFarland).

Sand is sterilized by heating to redness, and while still warm placed in test-tubes, which are then plugged.

The tube and its contents, the ends having first been plugged with cotton, are sterilized in a hot-air oven at 150° C.

One end of the tube is then fitted with a rubber cork through which passes a glass tube, which is connected with an aspirator (a hand-pump with a known capacity).

If 100 liters of air pass through the tube in fifteen minutes, the germs should all be arrested in the first sand-filter. And when the filters are removed, each filter for itself, and thoroughly mixed with gelatin, there should be no colonies developed from the second filter, *i. e.*, the one nearest the aspirator.

Sedgwick-Tucker Method.—A special form of tube is used, called an *aërobioscope.* It consists of a neck 2.5 cm. in length, an expanded portion 15 cm. long, and a long narrow tube of 15 cm. After sterilization the tube is partly filled with granulated sugar, which is the filtering material. By means of a vacuum gage and an air-pump, or ordinary aspirating bottles, the volume of air passing through the apparatus can be determined. After the air has been passed through, the sugar is gently shaken from the narrow tube into the expanded portion, and 20 c.c. of liquefied gelatin is poured in. The sugar dissolves, and the mixture is then rolled on the inner side of the glass as an Esmarch tube. This part of the apparatus is divided into squares to make the counting of colonies easy. The aërobioscope is very highly recommended.

Varieties Found in Air.—The only *pathogenic bacteria* found with any constancy are the Staphylococcus aureus and citreus; but any bacterium can, through accident, be lifted into the atmosphere, and under certain conditions may be always present—the Bacillus tuberculosis, for example, in rooms where consumptives are living.

Typhoid fever, influenza, pneumonia, and diphtheria may be conveyed through the air by the cough and expectoration of affected persons.

Non-pathogenic.—The micrococci predominate. Sarcinæ, yeasts, and molds constantly contaminate cultures.

In the ordinary habitations the average number of germs to the liter of air does not exceed five.

Around water-closets, where one would imagine a great number to exist, but few will be found, owing to the undisturbed condition of the air.

Sewer air seldom, if ever, contains bacteria, and neither typhoid fever, malaria, nor diphtheria has ever been traced to the escape of so-called *sewer-gas*.

Examination of Water.—The bacteriologic examination of water is today of as much importance as the chemical analysis, and must go hand in hand with it.

A water containing thousands of germs to the cubic centimeter is far less dangerous than one containing but two germs, if one of these two be a typhoid bacillus. It is not the number that proves dangerous, it is the kind.

If a natural water contains more than 500 germs to the cubic centimeter, it were well to examine its source, and consider it with suspicion.

As a means of diagnosis the examination is of but little use. An epidemic of typhoid fever occurs, the water is suspected, an examination is undertaken; but the period of incubation and the days passed before the water is analyzed have given the typhoid germs, if any had been present, ample time to disappear, since in water that contains other bacteria they live a few days only. Again, the water tested one day may be entirely free and the next day contain a great number, and before the typhoid germ can be proved to be present in that particular water the epidemic may be past. *Human sewage contamination* is determined by finding the *colon* bacillus, and if this is found in the course of an epidemic of typhoid the water containing it may well be suspected as being the cause.

Purity of Waters.—The purest water we have is the natural spring-water—water that has slowly filtered its way through various layers of gravel and sand and comes finally clear and sparkling from the ground. It is free from bacteria, but let such a water stand walled up in cisterns or

wells, or run through the wood, gathering the washings from pastures and farm lands, it becomes, as surface water, open to all sorts of impurities, and the bacterial nature of it changes every moment.

Artesian or Driven Well.—The *driven well* will secure to a certain extent a pure water. It is the only form of well or cistern that will insure this, since the water does not become stagnant in it; but it may connect with an outhouse—the soil being very loose—and thus bacteria and refuse water find their way into the well. The casing may not be water-tight and surface water can be sucked in.

Filtered Water.—Dangerous as surface water is, the greater quantity used is such, the inhabitants of larger towns and cities using chiefly the rivers and other large waters which course near them for drinking purposes. A purification or filtration can, to a certain extent, render these waters harmless.

Filtration is carried on on a large scale in the water-works of cities and towns, and bacteriologic examination is here of great service to determine if a water which has been filtered and may have a very clear appearance, and give no harmful chemical reaction, is entirely free, or nearly so, from germs; in other words, if the filter is a germ-filter or not; daily tests are necessary in order to insure safety, and if it is performing this function regularly, a good filter plant should show 99.8 per cent. efficiency, removing nearly all the bacteria.

Filter Materials.—When waters are muddy or when rapid filtration is wanted, mechanical filters are employed. The water is first treated with coagulants, like *alum*, which forms a flocculent precipitate and carries down with the suspended matter much of the bacterial content. This is then filtered through sand and gravel. Sedimentation and filtering slowly through gravel and sand is known as the slow process; the other as the rapid, filtration.

Charcoal sponge and asbestos, the materials formerly in use, are objectionable because germs readily develop on them and clog them, so that they require frequent renewal. In

very large filters, sand and gravel give the best results; the number of bacteria in a cubic centimeter is reduced to forty or fifty and kept at that number. This is a very pure water for a city water, though, as we stated before, not a safe one, for among those forty germs very dangerous ones may be found. It is then necessary for the users to refilter the water, before drinking it, through a material which will not allow any germs to pass, or, in the presence of an epidemic, to boil all water used for drinking purposes.

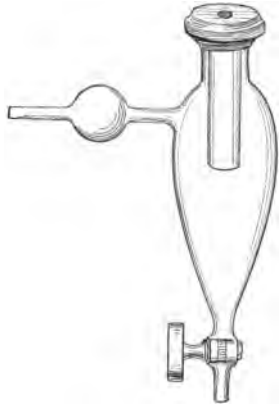


Fig. 117.—Flask fitted with porcelain bougie for filtering large quantities of fluid.

Pasteur-Chamberland Filter.—This very perfect filter consists of a piece of polished porcelain in the form of a cylinder closed at one end and pointed at the other. It is placed in another cylinder of glass or rubber, and the pointed portion connected with a bottle containing the water, or directly with the faucet of the water-pipe. The water courses through the porcelain very slowly and comes out nearly free from germs; pipe-clay, bisque, infusorial earth, and kaolin are also good filters. The only disadvantage is the long time it takes for the water to pass through. Pressure in the form

of an aspirator or air-pump is used to accelerate the passage.

These porcelain cylinders can easily be sterilized and the pores washed out.

All the cylinders or bougies are not germ proof, so that they must be tested, and most of them must be cleaned every fourth day. In recent years a number of organisms have been suspected of being so minute as to pass through a Berkefeld or Pasteur filter. At least the poison or virus is filterable, and, therefore, we cannot regard these as absolutely safe.

Boiling as a Means of Purifying.—The only safe measure in times of epidemics and with waters of unknown composition is **boiling,** not only of the drinking water, but all water used for domestic purposes; and this should especially be done in times of typhoid and cholera epidemics.

Varieties Found in Water.—The usual kinds found are non-pathogenic, but, as is well known, typhoid, cholera, and dysentery are principally spread through drinking-water, and many other germs may find their way into the water. Some of the common varieties give rise to fluorescence or produce pigment.

Eisenberg gives 100 different varieties as ordinarily found. Other intestinal diseases besides those mentioned above are supposed to be water borne. Diarrheas in epidemic form may come from suddenly changing a public supply, and the presence of the Bacillus coli communis means sewage contamination or fecal contamination; such contamination may come from the droppings of birds or other animals and need not necessarily imply human sewage, but 10 colon bacilli in 1 c.c. water is a serious pollution. Ice supplies require the same supervision as water supplies, for many bacteria, like the typhoid bacillus, retain their vitality for weeks after freezing.

Method of Examination.—(*After that suggested by the American Public Health Association, 1912 report.*)—Since the germs rapidly multiply in stagnant water, an examina-

tion must not be delayed longer than possible after the water has been collected. Every precaution must be taken in the way of cleanliness to prevent contamination; sterilized flasks with glass stoppers, pipets, and plugs must be at hand, glassware sterilized in autoclave at 120° C. for fifteen minutes, or dry heat at 160° C. for one hour, and the gelatin tubes or agar *dishes* be inoculated on the spot. If this cannot be done, the sample should be packed in ice until it arrives at the laboratory. If it is necessary to send the sample by rail, the bottle containing the sample should be wrapped in sterilized cloth, or the neck covered with tinfoil and the bottles placed in tin boxes (about 4 ounces—100 c.c.—is sufficient for bacterial analysis), and then packed in cotton or paper to prevent breakage and surrounded by plenty of ice until it reaches its destination. As soon as it arrives at the laboratory the sample is placed in a sterilized glass flask, and the flask then closed with a sterile cotton plug. A sterilized pipet is then dipped into the flask, and 1 c.c. of the water withdrawn and added to a Petri dish. To a second dish, a dilution of 1 c.c. of the sample with sterile distilled water is added, and other dilutions made if desired. To each plate 10 c.c. of standard agar at a temperature of 40° C. is added. Mix the water and media thoroughly by tipping the dish back and forth, and place in incubator at 37° C. for twenty-four hours. The incubator should be in a dark, well-ventilated, and moist place. Then count all the colonies present on each plate, which will give the number per cubic centimeter.

Water that is very rich in germs requires dilution with sterilized water fifty to one hundred times. Fewer colonies will be found on agar than on gelatin, even at the same temperature.

Special Media and Preparation.—In the preparation of media for water analysis, sodium chlorid must not be used. The reaction of most culture-media should be +1 per cent. to phenolphthalein.

Sugar broths should be *neutral*, and must be sterilized care-

fully in steam and not overheated, so as to prevent inversion of the sugar.

Examination for Bacillus Coli and Sewage Bacteria.—Instead of examining for typhoid bacilli, sewage contamination is best indicated by the presence of the colon group of organisms, although their abundance rather than mere presence is to be considered. There are many closely related bacteria which give reactions similar to the *Bacillus coli*, but they are chiefly of fecal origin, and for practical purposes they can be included in the colon group.

General Characteristics of Colon Group.—1. Fermentation of dextrose and lactose with gas-production. 2. Short bacillus, non-liquefying, Gram negative.

The committee of the Public Health Association recommends the following procedure:

Two Methods.—Method a.—Applicable for sewage waters. Preparation of an agar plate with a known volume of water, using lactose litmus-agar and incubating at 40° C. Bacillus coli will show its presence by red colonies (acid fermentation of the sugar); further testing is then needed to fully identify. Not all red colonies Bacillus coli.

Method b.—Cultivation, at 40° C., of a measured quantity of water in a fermentation tube containing a sugar broth. If gas appears, a portion of the liquid is plated as in method *a*.

Additional Details.—If in twenty-four hours *no red colonies* appear in the agar-lactose litmus Petri dishes, *Bacillus coli* is considered *absent*, providing the sample was a polluted one, so that the bacilli, if present, would be in a concentrated form. Only 1 or 2 c.c. of water can be used, because the ordinary water-bacteria spread rapidly and contaminate the other bacteria.

If acid-forming colonies are found, five or six are fished for subcultures on slanted agar, in fermentation tubes, milk, gelatin, peptone solution, and nitrate broth.

If the water is not strongly contaminated, an underground water, for instance, or a mountain stream, the better way is to inoculate two or three lactose or dextrose bouillon fermen-

tation tubes and place in an incubator at 40° C. *Note the presence of gas*, if any, at the end of twelve, twenty-four, thirty-six, and forty-eight hours. *If no gas forms, sewage bacteria are absent.*

If gas forms, plate at once a portion of the sediment as above on lactose litmus-agar. Test the other fermentation tubes for acidity, and the nature of the gas, whether any, and how much is absorbed by a 2 per cent. solution of sodium hydroxid. *Bacillus coli* should produce *between 30 and 70 per cent. of gas*, of which about *one-third is* CO_2 and is absorbed by the alkali; the remainder is hydrogen. The other broth culture can be tested for the presence or absence of *unfermented sugar* by Fehling's solution.

Diagnostic Points of Colon Bacillus.—*Microscopic.*—Non-spore-bearing motile bacillus.

Gelatin.—Non-liquefactive.

Dextrose Broth.—Fifty per cent. gas; one-third absorbed, CO_2; two-thirds, hydrogen.

Milk (litmus) coagulated in forty-eight hours and rendered acid; litmus colored red.

Peptone Solution.—Production of Indol.—(A peptone solution tube is inoculated with the culture and kept together with a control four days at 37° C. Then 2 drops of concentrated sulphuric acid and 1 centimeter of a 0.01 per cent. solution of sodium nitrate are added. The appearance of a pink color at the end of thrity minutes denotes the presence of indol.)

Presumptive Test.—If a water from a well or spring produces gas in the sugar broth and forms acid colonies on litmus-lactose agar, the presumption is strong that there is sewage contamination. If gas-production continues in a series of samples carefully collected for several days or weeks, there can be no doubt of a contamination, and especially if the well or spring is protected from surface water. Algæ which grow in service pipes, reservoirs, and deep wells may give rise to non-acid gas fermentation, but all well-water that, without further testing, forms acid colonies on litmus-agar lactose plates and ferments sugar broth, is open to suspicion, and if

there is evidence of the presence of typhoid fever or diarrheal diseases, the water should be boiled and subjected to careful analysis daily. There may be serious contamination and the chemical tests show no appreciable increase in the chlorids.

Bile Media.—In recent years bile salts or fresh bile mixed with lactose have been extensively used, as the bile inhibits the action of many bacteria and allows the colon and typhoid group to develop readily.

The *Jackson bile media* (see formula for media, Chap. X) is placed in fermentation tubes of 40 c.c. capacity, and inoculated with varying proportions of the water to be tested. Incubated at 37° C., and presence of gas looked for in twelve hours, twenty-four hours, and forty-eight hours, and the quantity and time noted.

In sewage and contaminated waters the lactose-bile gives better results than any other medium.

The Presumptive Test (Modified).—Plant $\frac{1}{10}$, 1, and 10 c.c. of water into liver broth tubes. Transplant from these into lactose bile in six and twelve hours. By using implantations of both lactose bile and liver broth, and then transplanting the liver-broth cultures into other lactose bile, we have in the original bile the vigorous *Bacillus coli.* The liver-broth dilutions give all the gas formers, strong and weak, and the difference between the original and the transplanted gives an idea of the attenuated *Bacillus coli* present. Thus all the gas formers are cultured.

Bacillus Typhosus.—By the use of bile media and other special media as enrichment and then transplanting on Hesse Agar, Conradi-Drigalski, or Endo media, the *Bacillus typhosus* are increased in number and the possibilities of diagnosing them made much easier. The Widal test is used to differentiate *Bacillus typhosus* from *Bacillus coli.*

Quantitative Tests.—The number of acid colonies in 1 c.c. and in 5 c.c. of water is taken as a measure of pollution, together with the total number of colonies of all bacteria present. Thus in 1 c.c. on the gelatin plate at 20° C. there may be

fifty colonies; on the agar plate at 37° C. ten colonies, five of which were acid-formers, or presumably Bacillus coli.

To count the colonies which develop upon the plates, a special apparatus has been designed, known as—

Wolfhügel's Counter.—A glass plate divided into squares, each a centimeter large, and some of these subdivided. This plate is placed above the dish with the colonies, and the number in several quadrants taken, a lens being used to see the smaller ones.

It is best to count all the colonies on the plate or dish.

Bacterial Treatment of Sewage.—Where sewage is to be rendered innocuous before being allowed to flow into streams, the process of nature has been imitated by the construction of septic tanks in which the sewage remains excluded from the air and subject to the action of the anaërobic bacteria present in the sewage. The organic nitrogen is reduced, and compounds of hydrogen and sulphur are formed. The effluent is then filtered through coke-beds, where the aërobic bacteria assist in further purification and over sand filters, or exposed to the air on *contact* beds. No method of sewage purification is very practical or safe. Pure water should not depend on the efficiency of sewage filtration, but should be obtained from a reasonably pure source.

Sewage is also treated by sedimentation with alum and filtration of the effluent over larger beds, or allowed to percolate through the soil, which is thereby enriched and utilized for agriculture. It is also dried and sold in a compressed form for fertilizer.

The Examination of the Soil.—The upper layers of the soil contain a great many bacteria, but because of the difficulty in analyzing the same, the results are neither accurate nor constant. The principal trouble lies in the mixing of the earth with the nutrient medium; little particles of ground will cling to the walls of the tube, or be embedded in the gelatin, and may contain within them myriads of bacteria. As with water, the soil must be examined immediately or very soon after it is collected, the bacteria rapidly multiplying in it.

When the deeper layers are to be examined, some precautions must be taken to avoid contamination with the other portions of the soil. One method, very laborious and not often practical, is to dig a hole near the spot to be examined and take the earth from the sides of this excavation.

Fränkel's Borer.—Fränkel has devised a small apparatus in the form of a borer, which contains near its lower end a small cavity, which can be closed up by turning the handle, or opened by turning in the opposite direction.

It is introduced with the cavity closed, and when it is at the desired depth, the handle is turned, the earth enters the cavity, the handle again turned, incloses it completely, and the borer is then withdrawn.

The earth can then be mixed with the culture-medium in a tube, and this gelatin then rolled on the walls of the tube after the manner of Esmarch, or it can be poured upon a plate, and the colonies developed therein.

Another method is to wash the earth with sterilized water, and the water then mixed with the culture-medium, as many of the germs are taken up by the water.

The roll-cultures of Esmarch give the best results, many of the varieties usually found being anaërobic.

Animals inoculated with the soil around Berlin are said to die almost always of *malignant edema*, and the soil of other towns produces *tetanus*. Many of the germs found are nitrogen formers and play a great rôle in the economy of the soil.

Bacteria and Soil Fertility.—*Nitrifying organisms* are found in the superficial layers of the earth. Organic matters found in sewage and in the fecal evacuations of animals form the basis for their activity, whereby nitrates, ammonias, and nitric acid result. The nitrogen necessary for the growing plant is thus produced. The nitromonas of Winogradsky belongs to this group. The soil tends to destroy ordinary disease-bacteria in a short time, but spores may remain dormant for a number of years, as, for instance, the spores of anthrax.

As bacteria are instrumental in transforming organic

matter, their influence in making the soil more useful for agricultural purposes has been the subject of much research. The richer the soil, the greater the number of bacteria. Most bacteria are found under the surface between 1 and 2 inches.

The rod-shaped organisms predominate.

From an agricultural standpoint the most important bacteria are those capable of liberating nitrogen and breaking up protein substance.

Carbohydrates are added to soil by manure, by the growth of grasses and crops, and these are decomposed by bacteria and methane and hydrogen produced.

Ammonia Production.—Most soil bacteria can produce ammonia; a few, the so-called urea bacteria, are capable of rapid transformation—*nitrification.*

Ammonia, oxidized into nitrites or nitrates, is possible through the agency of a group of micro-organisms given especial prominence by Winogradski. Moisture conditions and the presence of lime and mineral carbonates influence the nitrifying organisms.

The character of the growing crop affects the accumulation of nitrates; legumes assimilate nitrogen more rapidly than non-legumes.

Denitrification.—The reduction of nitrates to nitrites and ammonia is accomplished by a number of bacteria. Nitrate reduction is of little importance in the field, but under excessive manuring it may become so. Bacteria play the important part of making available to vegetation the nitrogen of the air.

Azofication.—Certain bacteria can fix *atmospheric nitrogen* and make it serve, but the energy necessary must be furnished by carbohydrates.

The enrichment of the soil by the growth of legumes has been shown to be due to the bacteria contained in the nodules or tubercles of the plant, these bacteria having the power to fix *nitrogen* and deriving their energy from the plant juices,

the plants in turn utilizing the nitrogen compounds created by the bacteria.

Soil Inoculation.—Artificial help to soils deficient in nitrogen-fixing organisms has been the subject of much experiment.

Nitragin.—Pure cultures of legume bacteria under the above name have been tried. Dried cultures under the name of *nitro-bacterine* have likewise been marketed, but neither of these methods has proved valuable; the matter is still in the experimental stage.

CHAPTER XXXII

BACTERIA IN MILK AND FOOD

The Bacteria of Milk.—Milk as secreted is sterile, but at every step in its passage from the cow to the consumer it is liable to contamination. Even the lower portion of the teat is a source of infection, owing to the presence of stagnated milk from the former milking, and, as milk ready for consumption usually contains thousands to millions of bacteria to the cubic centimeter, sterilization or pasteurization and supervision of the dairies should always be enforced for milk used for infant feeding.

A standard milk should be free from pus and should not contain more than 10,000 bacteria to the cubic centimeter.

Leukocytes are normally found in milk, and only when their number exceeds one million and pyogenic organisms are also present can pus be said to exist. Pasteurization of unclean milk sometimes renders it more dangerous as a food than untreated milk, because, by preventing the action of lactic-acid formers, other bacteria are permitted to develop and produce pathogenic toxins.

Pure Milk.—A pure milk is one that is obtained from a healthy cow, well groomed, in a clean room, by a healthy, clean person, in clean cans or bottles, and transported to the

consumer in as short time as possible without further handling, keeping the container in the mean time at a low temperature and protected from the air. *Such treatment is safer than any form of sterilization.*

Classification of Milk.—(*Abstract of resolutions adopted by the Commission on Milk Standards at Richmond, Va., May 2–3, 1913.*):

Milk shall be divided into three grades, which shall be the same for both large and small cities and towns.

GRADE A.—*Raw milk.*—Milk of this class shall come from cows free from disease as determined by tuberculin tests and physical examinations by a qualified veterinarian, and shall be produced and handled by employees free from disease as determined by medical inspection of a qualified physician, under sanitary conditions such that the bacteria count shall not exceed 100,000 per cubic centimeter at the time of delivery to the consumer. It is recommended that dairies from which this supply is obtained shall score at least 80 on the United States Bureau of Animal Industry score card.

Pasteurized Milk.—Milk of this class shall come from cows free from disease as determined by physical examinations by a qualified veterinarian and shall be produced and handled under sanitary conditions such that the bacteria count at no time exceeds 200,000 per cubic centimeter. All milk of this class shall be pasteurized under official supervision, and the bacteria count shall not exceed 10,000 per cubic centimeter at the time of delivery to the consumer. It is recommended that dairies from which this supply is obtained should score 65 on the United States Bureau of Animal Industry score card.

The above represents only the minimum standards under which milk may be classified in grade A.

GRADE B.—Milk of this class shall come from cows free from disease, as determined by physical examinations, of which one each year shall be by a qualified veterinarian, and shall be produced and handled under sanitary conditions such that the bacteria count at no time exceeds 1,000,000 per

cubic centimeter. All milk of this class shall be pasteurized under official supervision, and the bacteria count shall not exceed 50,000 per cubic centimeter when delivered to the consumer.

It is recommended that dairies producing grade B milk should be scored and that the health departments or the controlling departments, whatever they may be, strive to bring these scores up as rapidly as possible.

GRADE C.—Milk of this class shall come from cows free from disease as determined by physical examinations and shall include all milk that is produced under conditions such that the bacteria count is in excess of 1,000,000 per cubic centimeter.

All milk of this class shall be pasteurized, or heated to a higher temperature, and shall contain less than 50,000 bacteria per cubic centimeter when delivered to the customer. It is recommended that this milk be used for cooking or manufacturing purposes only.

Whenever any large city or community finds it necessary, on account of the length of haul or other peculiar conditions, to allow the sale of grade C milk, its sale shall be surrounded by safeguards such as to insure the restriction of its use to cooking and manufacturing purposes.

Classification of Cream.—Cream should be classified in the same grades as milk, in accordance with the requirements for the grades of milk, excepting the bacterial standards, which in 20 per cent. cream shall not exceed five times the bacterial standard allowed in the grade of milk.

Ice Cream.—Made and handled under sanitary conditions it contains mostly *Bacillus lactis acidi* type, not dangerous; but if made from milk and cream containing putrefactive bacteria, freezing will not prevent further growth and bacterial poisons may be developed, causing sickness and death. An examination of specimens collected gave as the lowest count 50,000 bacteria per cubic centimeter, and the highest 150,000,000 per cubic centimeter.

SOME BACTERIA FOUND IN MILK

Fermentation of Milk.—*Lactic Acid Lactose.*—Fermentation of milk is due to the conversion of milk-sugar into lactic acid. This can be accomplished by a number of different bacteria, such as Bacillus coli, streptococci and staphylococci, which are apt to be present about the dairy. The lactic-acid bacteria are commonly present in sour milk, and are chiefly concerned with fermentation. There are several varieties, but principally three groups.

The first group, like the Streptococcus pyogenes, is called the *Bacterium lactis acidi group.* Milk is curdled within twenty-four hours without gas-formation. The milk has a mild acid taste and agreeable odor. The curd is even, a true lactic fermentation.

The second group resembles the Bacillus coli—*Bacillus lactis aërogenes.* Indol and hydrogen sulphid often formed. Milk curdles, but the curd shrinks. Not easily emulsified. This fermentation undesirable.

The third group, true lactic bacteria—*Bacterium bulgaricum;* exclusively lactic acid; curd easily broken.

Bacterium Acidi Lactici (Hüppe).—Belongs to the same group as the Bacillus coli communis (see page 134).

Synonyms.—Bacillus acidi lactici; B. lactis aërogenes (Escherich).

Origin.—In sour milk.

Form.—Short thick rods, nearly as broad as they are long, usually in pairs, resembling *B. coli.*

Properties.—Immotile. Does not liquefy gelatin. Breaks up the sugar of milk into lactic acid and carbonic acid gas, the casein being thereby precipitated. The fermentation of milk produced by this group is offensive; taste undesirable. Curd is firm.

Stain.—Does not take Gram.

Growth.—Rapid and abundant; is facultative anaërobic. Grows at 10° C. Grows in all media and in absence of carbohydrates.

Stab-culture.—A thick dry crust with cracks in it forms on the surface after a couple of weeks.

Attenuation.—If grown through successive generations, it loses power to produce fermentation.

Streptococcus Acidi Lactici (Grotenfeld) (1889).—Widely distributed in nature.

Synonyms.—*Bacterium lactis acidi; Bact. Güntheri.*

Origin.—In sour milk.

Appearance.—Very short cells, often as large as oval cocci, in pairs or small chains, outer ends pointed.

Properties.—Immotile. Stain with Gram. Growth best at 30°–35° C.

Growth.—Facultative anaërobic. Delicate, opaque, resembling dewdrops. Bouillon containing glucose grows cloudy. Gelatin not liquefied. Milk coagulated. Strong acid reaction. Curd is soft and easily mixed within twenty-four hours. Gas is not formed.

In lactose-agar stab no surface growth, but all along the line.

Potato.—Scant growth.

Origin.—Almost always in sour milk, and the chief cause of lactic acid formation. Found at times in combination with B. acidi lactici and other bacteria. Sauer-kraut fermentation is due to streptococcus of lactic acid and yeasts, the latter producing gas.

Bacterium Bulgaricum.

Synonym.—*Bacterium caucasicus* (*v. Freudenreich*).

Origin.—Present in milk. Thought to be a product of eastern countries, but now recognized as universal. Arises from alimentary tract.

Properties.—Produces large amount of acid at higher temperature; non-motile.

Form.—Slender rods, 2 μ to 4 μ long, tending to form threads.

Staining.—Gram positive.

Growth.—Best growth at 40° C. Very meager colonies, hardly visible. Curdling homogeneous, changed later into soluble products. Gelatin not liquefied. Used to produce artificial buttermilks.

Potato.—Growth wrinkled and many-folded, gray changing to brown, extending over the entire surface as a thick covering or skin.

Agar Stroke.—Abundant, grayish, fatty, later on wrinkled skin.

Gelatin Stab.—On surface, grayish, fatty exudate covered with skin which slowly sinks as the media liquefy. Gelatin liquefied. No gas in sugar bouillon; acid is formed; no indol. Has been found in ropy or gelatinous bread and is considered the cause.

Bacillus Butyricus (Hüppe).—This bacillus causes butyric-acid fermentation. Supposed to be identical with Bacillus mesentericus.

Bacillus Amylobacter (Van Tieghem).—*Synonyms.—Clostridium butyricum (Prasmowsky); Vibrion butyrique of Pasteur; Bacterium saccharobutyricus (Klecki)* (Fig. 118).—*Origin.*—Found in putrefying plant-infusions, in fossils and conifera of the coal period, in cheese, water, earth.

Fig. 118. — Bacillus amylobacter.

Form.—Large, thick rods, with rounded ends, often found in chains. A large glancing spore at one end, the bacillus becoming spindle shaped in order to allow the spore to grow; hence the name, clostridium.

Properties.—Very motile; gases arise with butyric smell. In solutions of sugars, lactates, and cellulose-containing plants and vegetables it gives rise to decompositions in which butyric acid is often formed. Casein is also dissolved.

A watery solution of iodin will give the starch reaction and color blue some portions of the bacillus; therefore it has been called *amylobacter.*

Growth in Glucose Agar.—Rapid at 37°. Small indefinite colonies with gas-bubbles. No growth in gelatin.

Bacillus Cyanogenes (*Bacterium Syncyanum*) **(Hüppe).**—*Origin.*—Found in blue milk.

Form.—Small narrow rods about three times longer than they are broad; usually found in pairs. The ends are rounded.

Properties.—They are very motile; do not liquefy gelatin. A bluish-gray pigment is formed outside of the cell, around the medium. The less alkaline the medium, the deeper the color. It does not act upon the milk otherwise than to color it blue.

Growth.—Grows rapidly, obligate aërobe.

Colonies on Plate.—Depressed center, surrounded by ring of porcelain-like bluish growth. Dark-brown appearance under microscope.

Stab-culture.—Grows mainly on surface; a nail-like growth. The surrounding gelatin becomes colored brown.

Potato.—The surface covered with a dirty blue scum.

Attenuation.—After prolonged artificial cultivation loses the power to produce pigment.

Staining.—By ordinary methods. Gram positive.

Red milk and **yellow milk** are due to other chromogenic organisms, as, for instance, B. erythrogenes.

Examination of Milk.—*American Standard.*—Some bacteria are found in all milk as ordinarily handled. *Streptococci and colon group*, when present, *always regarded with suspicion.* A high-cell leukocyte count, when accompanied by chain bacteria, is an indication of udder disease. There should be several samples taken one week apart and an average made. Bacteria present may be counted in one of three ways.

Stewart-Slack Method.—Centrifuge 1 to 2 c.c. of milk; smear sediment on slide, and stain with Jenner or Wright stain and count bacteria in field.

Prescott-Breed Method.—In a special capillary tube $\frac{1}{100}$ c.c. of milk is sucked up and spread over a square centimeter on a microscopic slide, dried and fixed with methyl-alcohol. Flood with xylol to dissolve fat, stain with methylene-blue or Jenner, and decolorize slightly with alcohol. Focus 15 mm. of the specimen and count bacteria and cells present.

Multiply by 5000. This equals the number in $\frac{1}{100}$ c.c. Count several fields and average the result.

The Plate Method.—Microscopic examination, while not to be relied upon wholly, gives valuable and quick information as to the general character of bacteria, their apparent number, the presence or absence of barn-dirt and chain bacteria. The microscopic count differs greatly from plate count, because dead cells as well as living are shown.

Certified milk should have less than 10,000 bacteria to the cubic centimeter. According to the average taken from a count of four specimens, a rating is given to the milk, and this rating is to be interpreted only as other conditions are considered, such as cleanliness of the cattle and stalls, and chemic composition and method of handling the product.

Temperature.—Milk kept at 10° F. or lower will not allow ordinary bacteria to develop to any considerable extent; kept at a higher temperature, bacteria develop rapidly.

Separating or centrifuging permits the bacteria to be concentrated, and top-milk and cream contain more bacteria per cubic centimeter than whole milk.

Time, an element.

Milk freshly drawn, under proper precautions, may contain but few bacteria, but in forty-eight to seventy-two hours on ice bacteria will increase enormously. Market milk as ordinarily found in cities may contain millions of bacteria per cubic centimeter.

Pasteurization.—Milk heated to 60° C. for twenty minutes is called pasteurized. This increases the keeping quality and tends to destroy the vegetative forms of pathogenic bacteria.

To kill lactic acid, the instantaneous method, higher temperature, a few seconds only for pathogenic organisms is required. Pasteurization is beneficial only when there are supervision and inspection of original supply.

Milk as Source of Contagion.—*Harmless Varieties.*—Sour milk contains the Bacterium lactis acidi and is not dangerous, and is even considered beneficial, as, for instance, buttermilk.

Neutral Forms.—Many species of air and chromogenic varieties found in milk have no pathogenic properties, neither do they affect the composition of the milk.

Injurious Organisms.—Human diseases, like typhoid, diphtheria, and scarlet fever, may be conveyed through milk, the infection coming from some one concerned in handling the particular supply. The milk acts as a favorable medium for the pathogenic organisms that accidentally find their way into it. Animals wading in infected water have infected the milk. Utensils washed in polluted water have been found to be the cause in some epidemics of typhoid. *Carriers*, persons who harbor the diphtheria and typhoid bacteria, but who are not affected with illness, may likewise start epidemics of a kind, especially if working about dairies. Bacteria may enter milk from the animal, as Bacillus tuberculosis from diseased udder. Infantile diarrheas from the putrefactive *Bacillus coli* group, streptococcic sore throat from udder disease, are other forms of disease originating in milk.

Butter and Cheese.—Butter is milk-fat separated by creaming and churning, and as such partakes somewhat of the bacterial nature of the milk from which it is derived. The flavor of butter is due to the character of the acid bacteria used in souring the milk. By eliminating the gas-forming bacteria and by keeping his starting cultures pure the butter-maker can control and develop flavors as easily as the wine-maker. Pure cultures of lactic acid are supplied to butter-makers and used in creameries to inoculate sweet cream and milk. Bacteria coming from unclean utensils, polluted water, or dirty milk undoubtedly affect the flavor and often produce a poor quality of butter. Disease bacteria are not often conveyed through butter, although it is claimed that *Bacillus tuberculosis* has been found in salted butter.

Cheese.—The fat and casein salts and sugar-of-milk separated by curdling from the bulk of soluble portion of milk constitutes *cheese.* The curdling is accomplished by acid bacteria normally in milk, so-called *acid curd cheeses*, or by

the use of rennet to form a curd, *rennet curd cheese*, to which all the important varieties belong. Milk for cheese should be free from *Bacillus coli* or other deleterious bacteria. The milk for cheese cannot be pasteurized as for butter.

Testing Milk for Bacillus Coli.—A sample of milk is incubated at 35° C. for a few hours, noting the curd, whether firm or soft and gassy.

Wisconsin Test.—Milk curdled by rennet; curd cut and drained and jars kept at 30° C. to 40° C. The curd should have clean acid odor and taste.

After the curd has been formed, the cheese is allowed to ripen, and this is due to acid-forming bacteria, which permit the pepsin in the rennet to act. Various molds, notably Penicillium and Oïdium lactis, are used to give certain foreign cheeses their characteristic flavor.

Condensed milk has few bacteria in it. The sugar and condensation heat tend to prevent further growth of microorganisms.

Concentrated unsweetened milk is a form of pasteurized milk which is reduced in volume one-fourth. It is not always sterile, and bacteria may develop in it if exposed to warmth and air.

Buttermilk and similar fermented drinks depend on *Bacillus lactis acidi* and added yeasts. Bacillus bulgaricus gives more acid and allows partial sterilization.

Foods as a Source of Infection.—Foods eaten after little or no cooking, such as fruits, salads, and the like, and also oysters, are possible sources of bacterial diseases, and the so-called ptomain poisoning observed after the consumption of ice-cream, sausage, canned meats, etc., is the result of the action of bacteria or their products.

Oysters and fish from sewage-polluted waters have produced typhoid. Vegetables grown in manured ground or sprinkled with polluted water may be a possible source of disease. The practice of exposing meats and other food to street dust and flies is no doubt responsible for some disease.

Alcohol and Vinegar Fermentations.—On grapes are to

be found all forms of air bacteria as well as molds and yeasts, some beneficial, some harmful. The acid of grape-juice destroys many of the harmful forms, but some persist and must be dealt with by the wine-maker.

The various yeasts produce alcohol from the sugar of the grape. Vinegar bacteria likewise form a small amount of acetic acid. The wine-maker's success lies in obtaining a clean, *unbruised grape, aiding the work of the wine yeasts, and preventing the injurious forms from working.* The grapes are crushed and the juice allowed to settle. Pure cultures of tested yeast are used as starters of fermentation.

Fermentation is regulated by burning sulphur, which inhibits the growth of molds and harmful bacteria. After fermentation is completed the wine is cleared and freed from all organisms and kept as nearly as possible in a sterile condition.

Beer.—The fermentation is produced by yeasts and with a mixture of grains. Barley or other grains which have been allowed to germinate produce malt. The malt contains the enzymes which change starch into sugar. Then, by boiling, the enzyme is destroyed and fermentation by yeasts is permitted. The yeasts in modern breweries are pure cultures.

Wild yeasts or lactic-acid bacteria may contaminate beer.

Alcohol, brandy, and whisky are likewise the product of yeast fermentation, some sugary substance furnishing the material.

CHAPTER XXXIII

BACTERIOLOGIC EXAMINATION OF THE ORGANS AND CAVITIES OF THE HUMAN BODY

THE body, on account of its constant contact with the surrounding air, is necessarily exposed to infection, and we would be likely to find on the skin and in the oral, anal, and nasal cavities the varieties of microörganisms commonly around us. Through the water and food the body is also contaminated, but some organisms by predilection inhabit the mouth, intestine, and other cavities, and form there a flora distinctly their own.

The Skin.—The majority of microörganisms met with on the skin are non-pathogenic, although underneath the nails and in the hair pus-forming microörganisms often occur, producing sometimes serious abscesses on other parts of the body.

In the sweat-glands and the sebaceous glands various organisms have been found. The Staphylococcus pyogenes seems to be present constantly.

In foul-smelling perspiration of the feet Rosenbach found microörganisms pathogenic for rabbits.

Micrococcus cereus albus and flavus, Diplococcus liquefaciens albus and flavus, Staphylococcus pyogenes aureus, and Streptococcus pyogenes are found underneath the nails.

In eczema, Diplococcus albicans tardus, Diplococcus citreus liquefaciens, Diplococcus flavus liquefaciens, and Ascobacillus citreus.

In colored sweat, Micrococcus hæmatoides, Bacillus pyocyaneus.

A diplococcus is found in acute pemphigus.

The lepra bacillus, the tubercle bacillus in lupus, and the typhoid bacillus in the eruption of typhoid fever are a few of the specific germs found on the skin during the disease stage.

Infection results through some damage of the superficial layers. The injury may be very slight—an expanded hair-follicle may suffice to permit entrance of suppurative organisms.

The Conjunctiva.—The micrococcus of trachoma, the Koch-Weeks bacillus, considered to be the specific cause of acute catarrhal conjunctivitis, or "pink eye," and the Bacillus xerosis, are special germs found on the conjunctiva; the other varieties of air- and water-organisms, and those usually present on the skin, are also found. Löffler's bacillus and the pneumococcus have been found in some forms of conjunctivitis. The Koch-Weeks bacillus is the most contagious.

A special diplobacillus, known as the bacillus of Morax-Axenfeld, produces a stubborn form of conjunctivitis.

The gonococcus is found in ophthalmia of the new-born.

The Mouth.—The mouth is a favorite seat for the development of bacteria. The alkaline saliva, the particles of food left in the teeth, the decayed teeth themselves, all furnish suitable soil for their growth.

Quite a number of germs have been isolated and their properties partly studied. Many have some connection with the production of caries of the teeth, as Miller has well shown in his careful studies. The Leptothrix buccalis, found in nearly all mouths, is a long chain or filamentous bacillus which stains blue with iodin. It was formerly considered the cause of tartar on the teeth.

The Spirillum sputigenum, Spirochæta dentium, Micrococcus gingivæ pyogenes, Bacillus dentalis viridans, Bacillus pulpæ pyogenes, micrococcus of sputum septicemia, and Micrococcus salivarus septicus are a few of the organisms cultivated by Miller and Biondi from the mouth and supposed to be separate varieties. Besides these, the pneumobacteria, diphtheria bacillus, and tubercle bacillus are often met with, the first two in the mouths of healthy persons. The expired air in quiet respiration is free from bacteria, but in coughing, sneezing, etc., large numbers of organisms are violently ejected and the atmosphere about tubercular

patients is often saturated with tubercle bacilli. The bacteria may enter the system from the pharynx to the tonsils and cervical glands by means of the lymphatics.

Ear.—In the middle ear of new-born infants no pathogenic organisms have been found, but quite a number of non-pathogenic ones. In affections of the ear the pneumobacillus and the Staphylococcus pyogenes are most frequent.

When the streptococcus is present in acute suppurations, there is great danger of mastoiditis. In chronic otitis the gas-forming bacteria, as well as Bacillus pyocyaneus, is often found.

Nasal Cavity.—The nasal secretion, containing as it does dead cells and being alkaline in reaction, forms a good soil for the growth of germs.

Diplococcus coryzæ, Micrococcus nasalis, Bacillus fœtidus ozænæ, Bacillus striatus albus et flavus, Bacillus capsulatus mucosus, and Vibrio nasalis are some of the organisms described by various observers.

Stomach and Intestine.—The secretion of the stomach is in its normal state not a favorable soil for the development of bacteria, yet some germs resist the action of the gastric juice and flourish in it. When the acids of the stomach are diminished in quantity or absent altogether, the conditions for the growth of bacteria are more favorable. The alimentary canal of the new-born infant is sterile, but in a few hours after birth microörganisms begin to appear.

Some gastric bacteria normally present are Sarcina ventriculi, Bacterium lactis aërogenes, Bacillus subtilis, Bacillus amylobacter, Bacillus megaterium.

The intestinal organisms are more numerous, and the mucous lining of the intestines and the secretions there present are favorable to germ-growth.

Bacillus geniculatus Boas considers a sign of carcinoma of the stomach, and is always present, he claims, when the contents contain lactic acid.

Some investigators consider digestion dependent on microbic activity, but experiments with animals have shown that

life and digestion can proceed in a perfectly sterile condition. Food and air sterilized will not develop bacteria in the feces.

In the feces of the young a great many bacteria have been found that are supposed to stand in close relation with the intestinal disorders common to nurslings. The majority of bacteria usually present in the intestines are non-pathogenic. The following varieties may be met with in the feces: Micrococcus aërogenes, Bacillus subtilis, Bacillus butyricus, Bacillus putrificus coli, Bacillus lactis aërogenes, Bacillus coli commune, Bacillus subtiliformis, and the bacteria of cholera, dysentery, and typhoid, besides many yeast-cells.

Genito-urinary Passages.—In vaginal secretion Bumm has been able to find a number of organisms, some of which closely resemble the gonococcus; thus, there is the Diplococcus subflavus, Micrococcus lacteus faviformis, Diplococcus albicans amplus, and the vaginal bacillus.

In the urethra of healthy persons bacteria are sometimes found, usually having entered from the air.

In the normal secretions around the prepuce a bacillus called the smegma bacillus has been discovered. The spirochæte of syphilis can be obtained from lesions about the genitalia.

From urethral pus a number of diplococci other than the gonococci have been isolated.

From the urine itself a great number of bacteria have been obtained, but mostly derived from the air, finding in the urine a suitable soil. The colon and typhoid bacilli gain entrance into the bladder, possibly by way of the urethra, and produce cystitis. In a larger number of typhoid fever patients the bacilli are found in the urine.

Microörganisms of the Blood.—Many of the bacteria described in this book are found in the blood of the animal infected; anthrax bacilli are always found in the blood.

When animals are subcutaneously injected with pneumococci they are found in large quantities in the blood. The diseases of a hemorrhagic nature affecting fowls and swine usually show the presence of bacteria in the vascular system.

Bacteria may be recovered from the blood in all forms of septic infection, such as general sepsis, malignant endocarditis, puerperal sepsis, and typhoid fever. Tubercle bacilli are rarely if ever obtained from the blood.

Staining Blood Specimens.—A drop of blood is spread on a cover-glass and stained with the ordinary dyes; but in order to eliminate the coloring-matter of the red corpuscles and bring the stained bacteria more prominently into view, Gunther recommends that the blood, after drying and fixing, should be rinsed in a dilute solution of acetic acid (1 to 5 per cent.). The hemoglobin is thereby extracted, and the corpuscles appear then only as faint outlines.

Instead of "fixing" by heat, Canon employs alcohol for five minutes, especially in staining for influenza bacilli, which have been detected in the blood.

Blood Cultures.—As large a quantity of blood as possible—never less than 10 c.c.—is taken from a superficial vein, the median basilic, for example, by means of a sterile antitoxin syringe, a small incision being made through the skin over the vein in order to avoid skin infection. The blood so obtained is immediately transferred to culture-tubes, where the organisms are allowed to develop, and are then studied in the customary manner.

CHAPTER XXXIV

GERMICIDES, ANTISEPTICS, AND ANTISEPSIS

SUNLIGHT, pure air, and ordinary soap and water are effective disinfectants. Too often the burning of chemicals and the dipping of hands into antiseptic solutions partake of the nature of religious sacrifice, and the more nauseous the odor, the more effective is the incense supposed to be. Much of the perfunctory fumigation by the boards of health after the

minor contagious diseases, instead of teaching the people a lesson, create a false impression of security, and permit them to neglect the commoner means of ordinary cleanliness because of this assumed virtue of fumigation. The whole subject of fumigation and quarantine regulation needs more careful investigation and study.

A *germicide* is an agent capable of destroying bacterial life.

An *antiseptic* solution or substance is one that can inhibit or prevent the growth of bacteria without necessarily destroying them.

A *disinfectant* must be germicidal.

A *deodorant* may have no germicidal or antiseptic properties.

Preservatives are substances which prevent fermentation, but they are not always germicides.

In considering the value of a germicide, the strength in which it acts is the main consideration. Some very weak chemicals will inhibit and destroy the growth of bacteria if used in sufficiently concentrated solutions. Some bacteria will die in an acid medium; others are destroyed by too much alkali. Some bacteria are very readily destroyed in pure cultures, but are resistant to a considerable degree in the body tissues. Again, a germicide may be ideal in laboratory experiments, but wholly impractical at the clinic.

A 1 : 300,000 solution of *mercuric chlorid* (corrosive sublimate) will prevent the development of anthrax spores, but a 1 : 1000 solution is needed to destroy them.

Germicides are tested by action in various dilutions or in gaseous form on threads impregnated with virulent and spore-forming organisms. The length of time is noted that it takes to destroy anthrax bacilli or pyogenic organisms.

The infected material is subjected to the solution and then inoculated on media and compared with control, or tested for virulence on animals. Spore-forming organisms are very resistant to the most potent agents.

Heat is perhaps the best general *germicide*. For all articles that can be subjected to boiling or the direct flame there is no safer agent.

Superheated steam, or steam under pressure, is now in general use in sterilizing surgical dressings and instruments, and requires less time than ordinary steam.

The salts of metals of high atomic weights come next in order. Bichlorid of mercury and cyanid of mercury are the most powerful of chemical germicides, but in the human body they can be used in dilute solutions only, and in contact with highly albuminous solutions, insoluble and inert albuminates are liable to form, lessening the germicidal value. A 1 : 200 solution combined with an acid like *citric* will destroy the spores of anthrax in one hour, but much weaker solutions will destroy the anthrax bacilli in the blood, and for all practical purposes a 1 : 2000 solution is sufficient, destroying bacterial life in a few minutes.

One per cent. solution soda lye, NaOH, kills most bacteria in a few minutes, and, therefore, hot soapsuds is quite effective as a germicide.

Phenol in 5 per cent. solution will destroy most of the bacteria in less than five minutes. *Tricresol*, a combination of cresols, has three times the disinfecting power of phenol.

Formaldehyd, in gaseous form or in a liquid spray, is a very efficient germicide, and from the fact that it is not destructive to fabrics or paper has come into general use as a disinfectant. In combination with potassium permanganate or in suitable generators it is employed in houses after infectious diseases. It has no effect on insects, and where it is necessary to destroy these, other agents, known as *insecticides*, must be used in connection with the gas. The gas should be in a moist state—from 6 to 16 ounces for an ordinary room are needed; the room should be made as air-tight as possible, and the gas evolved as speedily as possible.

In the permanganate method 8 ounces (by weight) of potassium permanganate crystals are placed in a large tin vessel ten times the capacity of the disinfectant used. One pint of formaldehyd solution is quickly poured over the crystals. Formaldehyd gas is thereby generated at once.

This will produce enough gas for disinfection of 1000 cubic feet.

Solid formaldehyd in the form of candles is useful for small rooms, and some health boards employ it exclusively.

Sulphur dioxid, or sulphurous acid gas, is a germicide and insecticide, and is much used in disinfecting ships after yellow fever and malaria. It is obtained by burning sulphur in a pan over water, and about 3 pounds to 1000 cubic feet are necessary.

Copper sulphate, 1 part to 1,000,000 of water, is effective in destroying algæ, and is useful in large reservoirs as a temporary disinfectant.

Alcohol, iodin, chlorin, potassium permanganate, hydrogen dioxid, the salts of silver, lead, and zinc, salicylic acid, boric acid, anilin dyes (methyl-violet and methylene-blue), naphthalin, and creosols are a few of the substances in use as antiseptics and germicides in surgery. Their power varies with the strength of the solution and all have limitations.

In surgical operations more dependence is placed today on securing and maintaining a germ-free or aseptic condition than on the attempt to destroy germ life by chemicals. The irritation of antiseptics in some instances prevents the natural body defenses (phagocytes) from acting, and in abdominal operations, where no pus has been encountered, the blood-serum is sufficient or normal salt solution is alone used.

Sterilization of Hands, etc.—It has been shown by elaborate experiments that the skin, the hair, and clothing harbor many bacteria, some of a pathogenic nature. The surgeon who is anxious to secure good results should carefully attend to his toilet; the use of operating gowns, rubber gloves, operating shoes, face guards is now universal. The toilet of the hands of the surgeon is as important as that of the field of operation, but with the use of rubber gloves the painstaking directions as to the employment of a half-dozen or more cleansing agents and germicides are no longer followed.

Soap is an efficient germicide, the lye being in most cases powerful enough to prevent the growth of germs.

Filtration.—In the laboratory, and on a larger scale in the management of water-works, filtration is a method of sterilization, acting as it does by mechanically separating bacteria from a solution.

General Measures for Disinfection.—For *discharges*—urine, feces, sputum, vomitus—solution of phenol, 5 per cent., also fresh milk of lime, 1 part lime to 4 parts water. *Lime is of value only when sufficient alkali present.* Blankets, woolen clothing, soiled handkerchiefs, linen, boiling in steam, formaldehyd gas, or hot-air exposure.

Articles of little value should be *burned.* Books can be subjected to formaldehyd vapor or immersed in *gasolene.*

The hands and body washed in strong soapsuds and then in 1 : 1000 mercuric chlorid solution.

Tincture of *iodin,* for the skin and hairy parts, painted over the field of operation, has come into vogue as a very efficient *antiseptic.*

Woodwork and floors should be washed with soapsuds and 1 : 1000 solution of mercuric chlorid, the room itself subjected to formaldehyd vapor.

Testing the Value of Disinfectants.—*Rideal-Walker Standard.*—For comparing one disinfectant with another, they are compared with phenol solutions of known strength in their action on a culture of some microörganism (the Bacillus typhosus is now used in most laboratories). A standard temperature of 20° C. has been adopted by the workers of the United States Hygienic Laboratory, and some changes have been made by them in the Rideal-Walker method, so that it is referred to as the "*Hygienic Laboratory Phenol Coefficient.*"

The medium is made of beef-extract, according to the American Health Association standard, and must have a reaction of +1.5 in test-tubes containing 10 c.c. each of the medium.

The organism is a twenty-four-hour-old filtered broth culture of the *Bacillus typhosus.* Temperature of cultures and dilutions must be brought up to 20° C.

One-tenth of a cubic centimeter of the culture is added to 5 c.c. of the disinfectant dilution. The phenol control is made of different dilutions, from 5 to 10 strengths being employed. The disinfectant to be tested is likewise diluted, depending on the solubility, etc. An accurately graduated pipet distributes $\frac{1}{10}$ c.c. of the culture to each one of the dilutions, both of the phenol control and the test, and the tubes are then shaken gently three times. At intervals of two and one-half minutes a platinum loopful (the loop 4 mm. in diameter) is transferred from each tube and planted in the tube of broth medium. The inoculated tubes are then placed in an incubator at 37° C. for forty-eight hours, and at the end of this time results are recorded.

The coefficient is determined and recorded as in the example here given.

EXAMPLE.

Name of disinfectant to be tested, A.

Temperature, 20° C.

Culture used, *Bacillus typhosus*, 0.1 c.c. to 5 c.c. disinfectant.

DILUTION	TIME EXPOSED IN MINUTES					
	2½	5	7½	10	12½	15
Phenol:						
1 : 80	—	—	—	..	..	..
1 : 90	+	—	—	—	..	..
1 : 100	+	+	+	—	—	—
1 : 110	+	+	+	+	+	—
Disinfectant:						
1 : 350	—	—	—	..	..	..
1 : 375	—	—	—	..	..	..
1 : 400	+	—	—	—	..	..
1 : 500	+	+	—	—	—	—
1 : 650	+	+	+	+	+	—

The weakest disinfectant dilution that kills within two and one-half minutes (1–375) is divided by the weakest phenol

dilution (1:80), thus $\frac{375}{80} = 4.69$, and the same is done for the strength that kills in fifteen minutes, namely:

$$\frac{650}{110} = 5.91$$

The average of these, $\frac{5.91+4.69}{2} = 5.30$, is called the coefficient. In other words, disinfectant A has a value of 5.30 times that of phenol. A disinfectant with a phenol coefficient less than 1 is of very low germicidal value.

CHIEF CHARACTERISTICS

PART I.—

This classification into non-pathogenic and pathogenic is not strictly correct, as special

NAME.	GENUS.	BIOLOGY.	PRODUCT
ACETI.	Bacillus.	Short motile rods in zoöglea; aërobic.	Ferment.
ACIDI LACTICI.	Bacillus.	Short, immotile rods; aërobic.	....
ACIDI LACTICI.	Streptococcus.	Short, immotile, oval cocci.	....
ACTINOBACTER.	Bacillus.	Immotile rods with capsule; facul. anaërob.	....
AËROGENES.	Bacillus.	Identical with B. acid lactici.	....
AËROPHILUS.	Bacillus.	Slender rods in threads; immotile; oval spores; aërobic.	....
AGILIS.	Micrococcus.	Mobile diplococci with fine flagella.	Red pigment.
ALBA.	Beggiatoa.	Cocci and spirals with sulphur.	....
ALBA.	Sarcina.	Small cocci in packets	White pigment.
ALBICANS AMPLUS.	Micrococcus.	Large cocci and diplococci.	....
ALBICANS TARDISSIMUS.	Micrococcus.	Diplococci colored by Gram.	....
ALBICANS TARDUS.	Micrococcus.	Diplococci not motile.	....
ALLII.	Bacillus.	Very small rods.	Alkaloid pigment.
AMYLIFERUM.	Spirillum.	Rigid spirilla with spores; turns blue with iodin.	....
AMYLOBACTER.	Bacillus.	See *Butyricum*, with	which it is identical.
AQUATILIS.	Micrococcus.	Very small cocci in irregular groups.	....
ARACHNOIDEA.	Beggiatoa.	Very thick filaments containing sulphur; motile.	....
ARBORESCENS.	Bacillus.	Thin rods, with rounded ends in threads, and singly; immotile.	Yellow pigment.
ATTENUATUM.	Spirillum.	Threads with narrowed ends.	....
AURANTIACA.	Sarcina.	Small cocci in pairs and tetrads; strongly aërobic.	Orange-yellow pigment.
AURANTIACUS.	Bacillus.	Motile, short thick rods, often in long threads.	Orange-yellow pigment.

OF THE PRINCIPAL BACTERIA.

NON-PATHOGENIC BACTERIA.

many of the non-pathogenic varieties have disease-producing properties under conditions.

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Not liquefy; membranous growth.	Produces acetic-acid fermentation.	Air.	Kützing.
Not liquefy; small white points, porcelain-like; slow.	Lactic-acid fermentation; precipitates casein.	Air; sour milk	Pasteur.
Growth faster than above appearance same.	Alcohol is formed after the lactic-acid fermentation.	Sour milk.	Grotenfeldt.
....	Causes fermentation with gas and alcohol.	Air.	Duclaux.
....	....	....	Miller.
Liquefy rapidly; small yellow-gray colonies.	....	Old cultures.	Liborius.
Slowly liquefying, forming a cone with rose-red color.	....	Drinking-water.	Ali-Cohen.
....	....	Sulphur springs.	Vauch.
Slow growth in small white colonies.	....	Air and water.	Zimmerman.
Slowly liquefy; gray colonies; growth fairly rapid.	Is colored by Gram's method.	Vaginal secretion.	Bumm.
Small white points, not liquefying; very slow growth.	....	Urethral pus.	Bumm.
Grows slowly on surface, the boundary raised; twice as large as above.	....	Skin in eczema.	Unna, Tommasoli.
Bright-green pellicle on agar.	Decomposes albumin.	Green slime of onions.	Griffiths.
....	....	Water.	Van Tiegham.
Light-yellow colonies; serrated edges.	....	Old distilled water.	Bolton.
....	....	Sulphur water.	Agardh.
Colonies, radiating from an oval center like roots; later on colored yellow; slowly liquefy.	....	London Water-works.	Frankland.
....	....	Stagnant water.	Warming.
Rapidly liquefy; little orange-yellow colonies, not growing in high temperature.	....	Air and water.	Koch.
Slowly growing; nail cultures; shining and orange-yellow; not liquefy.	....	Water.	Frankland.

NON-PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
AURANTIACUS.	Micrococcus.	Oval cocci in pairs and singly; immotile.	Orange-yellow pigment in water, alcohol, and ether; insoluble.
AUREA.	Sarcina.	Cocci in packets.	Golden-colored pigment; soluble in alcohol.
AUREUS.	Bacillus.	Straight motile rods lying parallel.	Golden-yellow pigment.
BALTICUS.	Bacillus.	Short rod.	Phosphorescence.
BIENSTOCKII.	Bacillus.	See *Putrificus, coli.*	
BIFIDUS.	Bacillus.	Slender diplococcus, pointed ends, nonmotile, anaërobic.	....
BILLROTHII.	Micrococcus (ascococcus).	Groups of cocci surrounded with capsule; zoöglea, aërobic.	....
BRUNNEUS.	Bacillus.	Motile rods.	Brown pigment.
BUTYRIC-ACID FERMENTATION.	Bacillus.	Large, slender motile rods in pairs; spores; facul. anaërobin.	Diastase.
BUTYRICUM (amylobacter).	Clostridium.	Thick motile rods enlarging for the spores; obligate aërobic.	Amyloid substance.
CÆRULEUS.	Bacillus.	Rods in long chains.	Blue pigment, not soluble in water, alcohol, or acid.
CANDICANS (candidus).	Micrococcus.	Masses of cocci.	....
CAROTARUM.	Bacillus.	Threads of rods that bend in various directions; oval spores	....
CATENULA.	Bacillus.	Motile rods with spores.	....
CAUCASICUS.	Bacillus.	Motile rods, with spores in each end.	....
CERASINUS SICCUS.	Micrococcus.	Very small cocci, singly and in pairs; aërobic.	Cherry-red pigment.
CEREUS ALBUS.	Micrococcus.	Cocci in short chains and bunches, colored by Gram.	....
CEREUS FLAVUS.	Micrococcus.	Staphylococcus and streptococcus, and in zoöglea, colored by Gram.	....
CHLORINUS.	Bacillus.	Large rods, motile, green-colored, due to chlorophyll; aërobic.	Green pigment, soluble in alcohol.

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Round orange-yellow colonies, mostly on surface; slow growth; not liquefying.	....	Water.	Cohn.
Liquefy; bright golden layer on potato.	....	Exudate of pneumonia.	Mace.
Slow-growing, chrome-yellow, whetstone in shape; not liquefy.	....	Water and skin of eczema.	Adametz and Unna.
Do not liquefy; require glucose for growth.	....	Baltic Sea.	Fischer.
Oval colonies after three days on glucose agar.	Feces of infants breast-fed.	....	Tissier.
Creamy layer on surface of gelatin.	....	Putrid broth.	Cohn.
....	....	Maize.	Schröter.
Liquefy rapidly; gray veil on surface of potato.	Casein precipitates and changed into butyric acid; ammonia set free.	Air.	Hueppe.
Not cultivated.	Forms butyric acid in presence of lactic acid.	Air, earth, and water.	Prazmowski and Van Tiegham.
Liquefy; a deep-blue layer on potato.	....	Water.	Smith.
Not liquefy; nail-shaped in test-tube.	....	Air around old cultures.	Flügge.
Rapidly liquefy on surface, a network center on potato; round, light gray; grow rapidly.	....	Cooked carrots and beets.	A. Koch.
....	Causes albumin to ferment.	Old cheese.	Duclaux.
....	Ferments milk, producing the kefir drink.	Kefir; grain.	Kern.
On potato; rapidly forming cherry-red scum, not developed on gelatin.	....	Water.	List.
Not liquefy; small, wax-like drops; thick gray layer on potato; growth rapid.	....	Pus.	Passet.
Not liquefy; dark-yellow colonies; wax-like appearance.	....	Pus.	Passet.
Liquefy; greenish-yellow colonies.	....	Water.	Engelman.

NON-PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
CHLORINUS.	Micrococcus.	Cocci in zoöglea.	Green pigment, soluble in alcohol and water.
CINNABAREUS.	Micrococcus.	Large oval cocci in pairs; aërobic.	Brown-red pigment; foul odor.
CITREUS.	Bacillus (ascococcus).	Straight and bent rods in bundles; motile.	Citron-yellow pigment.
CITREUS.	Micrococcus.	Large round cocci in chains of eight and more.	Cream-colored pigment.
CITREUS CONGLOMERATUS.	Micrococcus.	Diplococci and tetrads; aërobic.	....
CLAVIFORMIS.	Bacillus (tyrothrix).	Small rods; spores; true anaërobin.	....
CLOACÆ.	See *Proteus*.		
CONCENTRICUM.	Spirillum.	Thick motile spirals with flagella; aërobic.	....
CORONATUS.	Micrococcus.	Cocci singly and streptococci; aërobic.	....
CORYZÆ.	Micrococcus.	Large diplococci with rounded ends, the contact surfaces flat.	....
CREPESCULUM.	Micrococcus.	Round and oval cocci, singly and in zoöglea.	....
CYANEUS.	Micrococcus.	Oval cells.	Blue pigment.
CYANOGENUS (blue milk).	Bacillus.	Motile rods in chains; spores; aërobic.	Alkali and a pigment deepened by acids.
DICHOTOMA.	Cladothrix.	Various forms—rods, spirals, and cocci, in long threads.	....
DIFFLUENS.	Micrococcus.	Oval cocci; aërobic.	Fluorescent pigment, soluble in water.
DISTORTUS.	Bacillus (tyrothrix).	Motile rods; spores; aërobic.	Alkali.
DYSODES.	Bacillus.	Long and short rods; spores.	An odor resembling peppermint and turpentine.
ENDOPARAGOGICUM.	Spirillum.	Dry motile spirals, joined in peculiar shapes.	....
ERYTHROSPORUS.	Bacillus.	Motile rods and threads; spores, slender.	Greenish-yellow pigment.
FIGURANS (mycoides).	Bacillus.	Large motile rods; spores; long threads; aërobic.	....
FILIFORMIS.	Bacillus (tyrothrix).	Short motile rods; spores in one end.	....

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Yellow-green layer on gelatin.	....	Boiled eggs.	Cohn.
Not liquefy; slow growth; bright-red points.	....	Air and water.	Flügge.
Slow growth; after two weeks small yellow points which take various shapes on potato; citron-yellow layer; growth more rapid.	....	Skin in eczema.	Unna and Tommasoli.
Dirty, cream-colored colonies, which are raised and moist.	....	Water.	List.
Lemon-yellow colonies.	....	Dust and blennorrhagic pus.	Bumm.
....	Ferments milk, giving rise to alcohol.	Fermenting albumin.	Duclaux.
Not liquefying; concentrically disposed colonies; very slow growth; not growing on potato.	....	Putrefying blood.	Kitasato.
A halo formed around the colonies.	....	Air.	Flügge.
White, raised glassy colonies, at first like pneumococci, later culture flattened; not liquefying.	No pathogenic action.	Acute coryzal secretion.	Hajek.
....	....	Putrefying infusions.	Cohn.
Bluish-green colonies.	....	Cooked potatoes.	Cohn.
Not liquefying; small white colonies.	Changes milk to deep-blue color.	Air of certain countries.	Fuchs.
Cultivated in infusion of plants.	....	Water.	Cohn.
Do not liquefy; small granular, yellow colonies; green fluorescence.	....	Air.	Schröter.
....	Milk made viscid and casein precipitated.	Air.	Duclaux.
....	....	Bread and yeast.	Zopf.
....	....	Trunk of worm-eaten tree.	Sorokin.
Does not liquefy; green fluorescence; white colonies.	....	Air and putrefying substances.	Cohn.
Liquefying; root-like processes extending in the gelatin; feather form in test-tube.	....	Garden-earth.	Flügge.
....	Causes casein to be precipitated from milk.	....	Duclaux.

NON-PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
FISCHERI.	Bacillus.	....	Phosphorescence.
FITZIANUS.	Bacillus.	Short rods in threads; spores as large as the rods.	....
FLAVA.	Sarcina.	Small cocci in packets.	Pigment.
FLAVUS.	Bacillus.	Small rods; immotile.	Pigment.
FLAVUS DESIDENS.	Streptococcus.	Cocci and diplococci in chains; aërobic.	Yellow-brown pigment.
FLAVUS LIQUEFACIENS.	Micrococcus.	Cocci and diplococci in zoöglea.	Pigment.
FLAVUS TARDIGRADUS.	Micrococcus.	Cocci in short chains, and diplococci.	Chrome-yellow pigment.
FLUORESCENS FŒTIDUS.	Micrococcus.	Small diplococci.	Blue-green pigment: acids turn red.
FLUORESCENS LIQUEFACIENS.	Bacillus.	Short motile rods; very thin.	Green fluorescent pigment.
FLUORESCENS NIVALIS.	Bacillus.	Short rods; motile.	Blue-green pigment.
FLUORESCENS PUTRIDUS.	Bacillus.	Motile rods; short, with rounded ends.	Green fluorescent pigment.
FOERSTERI.	Cladothrix.	Threads twisted in spirals; very irregular.	....
FŒTIDUM.	Clostridium.	Rods of varying length; very motile; a large spore in one end; anaërobic.	Strong gas-production; very foul odor.
FŒTIDUS.	Micrococcus.	See *Crepesculum*, with	which it is identical.
FUSCESCENS.	Sarcina.		
FULVUS.	Micrococcus.	Round cocci.	....
FUSCUS LIMBATUS.	Bacillus.	Short rods; very motile; facultatively anaërobic.	Brown pigment.
FUSIFORME.	Bacillus.	Spindle-shaped, with pointed ends.	....
GENICULATUS.	Bacillus (tyrothrix).	Rods variable length; spores.	A bitter substance.
GIGANTEUS URETHRÆ.	Micrococcus.	Streptococci in thick knots.	....
GRASS. See *Timolhy*.			
GRAVEOLENS.	Bacillus.	Small rods, nearly as broad as they are long.	Foul gas.
HÆMATODES.	Micrococcus.	Cocci in little zoöglea.	Red pigment.

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Not liquefying; requires peptone for growth.	....	....	Beyerinck.
Transparent on surface; dark center in the deep; not liquefying.	Produces ethylic alcohol in meat extract.	Unboiled hay-infusion.	Zopf.
Liquefying.	....	Vomited matter.	de Bary.
Liquefying; yellow viscid colonies; foul odor.	....	Drinking-water.	Mace.
Yellow porcelain-white colonies.	....	Air and old cultures; water.	Flügge.
Liquefying rapidly; yellow colonies.	....	Air and old cultures; water.	Flügge.
Softens gelatin; yellow beads, isolated.	....	Air.	Flügge.
Little button-like colonies that later on sink in, surrounded by violet-green color; liquefying; growth rapid.	....	Post-nasal space.	Klamann.
Liquefying; white, sunken, iridescent colonies.	....	Water and air; conjunctival sac.	Flügge.
Quickly liquefying; growth rapid; small white points; later on, surrounded by blue-green fluorescence.	Colors the glacial waters green.	In snow and ice of Norway.	Schmolck.
Not liquefying; transparent at first, then green fluorescence and urinary odor.	....	All putrefactions.	Flügge.
....	....	Lacrimal canal.	Cohn.
Liquefying; growth rapid; small colonies that soon become filled up with fluid and assume a spherical form.	....	Old cheese and serum of mice inoculated with garden-earth.	Liborius.
Conic rusty-red colonies.	....	Excrement of horse.	Cohn.
Small brown colonies, along needle-track little branches; not liquefy.	....	In foul eggs.	Scheibenzuber.
....	....	Spongy layer on sea-water.	Warming.
....	....	Air and milk.	Duclaux.
No growth on gelatin; on agar, thin drops; nearly transparent; very slow growth; in bouillon, a flaky precipitate.	....	Normal urine and urethra.	Lustgarten.
Liquefying; irregular grayish, later greenish, colonies, with very foul odor.	....	Skin between toes.	Bordoni-Uffreduzzi.
Grows best on white of egg at 37° C.; red layer.	....	Sweat of man.	Zopf.

NON-PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
HANSENII.	Bacillus.	Medium large rods.	Yellow pigment; insoluble.
HAY. See *Subtilis*.			
HOFFMAN'S. See	*Pseudodiphtheri*	*a*.	
HYACINTHI.	Bacillus.	Short rods in dumb-bell shapes.	....
HYALINA.	Sarcina.	Round cocci in groups of 4 to 34.	....
IANTHINUS.	Bacillus.	See *Bacillus violaceus*.	
INDICUS.	Bacillus.	Short, motile rods; no spores; anaërobin facul.	Scarlet pigment altered by heat.
INTESTINALIS.	Sarcina.	Very regular packets of cocci, eight in each.	....
JEQUIRITY.	Bacillus.	Medium-sized rods; spores.	Ferment called abrin.
KÜHNIANA.	Crenothrix.	Long threads, breaking up into cocci. They are ensheathed.	....
LACTEUS FAVIFORMIS.	Micrococcus.	Diplococci; not decolorized by Gram.	....
LACTIS ERYTHROGENES.	Bacillus.	Short immotile rods; round ends.	Yellow pigment and red pigment.
LEPTOMITIFORMIS.	Beggiatoa	Filaments medium size.	....
LEUCOMELÆNUM.	Spirillum.	Two or three spirals; dark granular contents; clear spaces between.	....
LINEOLA.	Bacillus.	Short motile rods in zoöglea, with flagella.	....
LIODERMOS.	Bacillus.	Short motile rods; rounded ends.	....
LITORALIS.	Merismopedia.	Cocci in groups of fours, containing sulphur.	....
LITOREUS.	Bacillus.	Oval rods, never in chains or zoöglea.	....
LIVIDUS.	Bacillus.	Medium-sized rods; motile.	Deep blue-black pigment.
LUTEA.	Sarcina.	Cocci singly and in fours.	Pigment citron-yellow.
LUTEUS.	Bacillus.	Short immotile rods, with large oval spores.	Pigment; soluble in water; acids intensify.
LUTEUS.	Micrococcus.	Oval cocci.	Pigment, not acted upon by acid or alkali.

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
On potato, a yellow growth which changes with age.	....	Yellow skin of nutrient infusions.	Rasmussen.
....	....	Slime of diseased hyacinth-bulbs.	Wakker.
....	....	Marshes.	Kützing.
Liquefying; oval colonies; scarlet-colored.	....	Intestine of monkey.	Koch.
....	....	Intestine of fowls.	Zopf.
....	Ferment causes ophthalmia.	Infusion of jequirity bean.	Sattler.
Colonies brick-colored from oxid of iron.	....	Drinking-water of wells.	Rabenhorst.
Not liquefying; white colonies; grow well on potato.	....	Mucus of vagina and uterus.	Bumm.
Small, round yellow dots, later on cup-shaped, with rose-colored periphery; liquefying.	....	In red milk and feces.	Grotenfeldt.
....	....	Sulphur waters.	Trévisan.
....	....	Water over rotting plants.	Perty.
Slimy layer on potatoes.	....	Stagnant water.	Müller.
Liquefying; transparent, then thick layer on potato; like gum.	....	Air and potatoes.	Flügge.
....	....	Sea-water.	Oersted and Rabenhorst.
	....	Sea-water.	Warming.
Ink-spot at first, slowly liquefying; blue-violet colored later on; slow growth.	....	Berlin Waterworks.	Flügge and Proskauer.
Not liquefying; little elevations; citron-yellow center; yellow layer on potato.	....	Air.	Schröter.
Not liquefying; irregular in form; golden-yellow colored.	....	Air.	Flügge.
Do not liquefy; small citron-yellow colonies on potato.	....	Air.	Schröter.

NON-PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
LUTEUS.	Micrococcus.	Diplococci very motile.	Yellow pigment, turning brown-red.
MAIDIS.	Bacillus.	Rods with pointed ends, very motile; seldom in threads; oval spores.	....
MARSH.	Spirillum.	See *Plicatile.*	
MEGATERIUM.	Bacillus.	Large motile rods; spores; aërobic.	....
MELANOSPORUS.	Bacillus.	Rods; aërobic.	Black pigment, not acted upon by acids or alkalis.
MERISMOPEDIOIDES.	Bacillus.	Threads of rods which are formed from cocci-like spores; zoöglea in packets.	....
MESENTERICUS FUSCUS (potato).	Bacillus.	Small motile rods with spores.	....
MESENTERICUS VULGATUS (potato).	Bacillus.	Thick motile rods in threads; spores.	Diastase.
MESENTEROIDES.	Leukonostoc.	Masses of cartilaginous zoöglea, composed of rods and cocci; arthrospores.	....
MILLER'S.	Bacillus.	Delicate rods, slightly curved; immotile.	....
MINUTA.	Sarcina.	Cube-shaped packets.	....
MIRABILIS.	Beggiatoa.	Very wide threads, rounded ends and curled; sulphur granules.	....
MULTIPEDICULOSUS	Bacillus.	Long, slender rods.	....
MYCOIDES. See	*Rasmosus.*		
NASALIS.	Micrococcus.	Diplococci, motile; also streptococci.	....
NAVICULA.	Bacillus.	Spindle-shaped rods.	Amyloid material.
NITRIFICANS.	Micrococcus.	Small cocci.	Forms saltpeter.
NIVEA.	Beggiatoa.	Very thin filaments.	....
NODOSUS PARVUS.	Bacillus.	Rods formed at angles; immotile.	....

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Round, light-yellow colonies, growing larger in a few days; on potato a slimy covering with moldy odor; slowly liquefying.	....	Water.	Adametz.
Gray points in deep, veil-like on surface; liquefying; on potato, a wrinkled skin of brownish color.	In solutions of sugar an aldehyd is produced.	In maize and in pellegra; feces.	Paltauf and Heider.
Yellow irregular masses; thick layer on potato.	....	Cooked cabbage.	De Bary.
First gray, then black, pellicle.	....	Air and potatoes.	Eidam.
....	....	Stagnant water.	Zopf.
Liquefying; white colonies, ray-like periphery brown layer on potato.	....	Potato.	Flügge.
Yellow colonies, dark center, ciliary processes at periphery; brown layer on potato, penetrating the substance.	Coagulates milk and forms diastase out of starch.	Air and old potatoes.	Flügge.
....	Converts molasses into a gelatinous mass.	Beet-root juice.	Cienkowski.
Liquefies; not growing on the surface.	....	Caries of teeth.	Miller.
Grows slowly; reacts to iodin, turning blue.	....	Sour milk.	De Bary.
....	....	Sea-water.	Cohn.
Insect-shaped colonies.	....	Potatoes.	Flügge.
Grayish points, raised, opaque; rapid growth; not liquefying.	....	Nasal space and secretion.	Hack.
....	....	Potatoes.	Reinke and Berthold.
....	....	Soil.	VanTiegham
White flakes.	....	Sulphur waters.	Rabenhorst.
Slow growth at 37° C.; in agar a white line, which in the center becomes porous.	....	Urethral secretion.	Lustgarten.

NON-PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
OBLONGUS.	Micrococcus.	Motile cocci, singly and in filaments; aërobic.	....
OCHROLEUCUS.	Micrococcus.	Cocci in pairs and packets; spores.	Yellow pigment.
PALUDOSA.	Sarcina.	Spheric, transparent, colorless cocci.	....
PASTEURIANUS.	Bacillus.	Differs from Bacillus aceti in that the cells contain an amyloid matter.	....
PFLÜGERI.	Bacillus.	Short rods in threads.	Phosphorescence.
PHOSPHORESCENS GELIDUS.	Bacillus.	Motile; round, short rods; aërobic.	Phosphorescence.
PHOSPHORESCENS INDICUS.	Bacillus.	Large motile rods.	Phosphorescence.
PHOSPHORESCENS, North Sea.	Bacillus.	Motile rods.	Phosphorescence.
PHOTOMETRICUS.	Bacillus.	Motile, red-colored rods.	Sulphur and red pigment caused by light.
PLICATILE.	Spirillum.	Long motile, thin spirals; round ends.	....
POLYMYXA.	Clostridium.	Motile rods in threads with spores.	Amyloid colored blue by iodin.
PRODIGIOSUS.	Bacillus.	Short motile rods; aërobic.	Red pigment, soluble in alcohol trimethylamin.
PROTEUS MIRABILIS.	Bacillus.	Very motile, short rods; aërobic.	....
PROTEUS VULGARIS.	Bacillus.	Rods sometimes curved as spirillum.	....
PROTEUS ZENKERI.	Bacillus.	Motile rods.	....
PSEUDO-DIPHTHERIÆ (Hoffman).	Bacillus.	Small rods, similar to the true bacillus; immotile.	....
PUTRIFICUS COLI.	Bacillus.	Slender motile rods; long threads; spores.	....
PYOGENES TENUIS.	Micrococcus.	....	....
RADIATUS.	Bacillus.	Motile rods with rounded ends; anaërobic oval spores.	Strong-smelling gas.

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Grows best in cultures to which glucose and ammonium tartrate have been added.	Causes gluconic fermentation.	Beer.	Boutroux.
Liquefying; slow growth; thin yellow membrane; sulphurous odor.	....	Urine.	Prove.
....	....	Water from sugar-factory.	Schröter.
....	....	Heavy beers.	Hansen.
Not liquefying; requires glucose; grows well on potato.	....	Putrid meat and fish.	Ludwig.
Not liquefying; grows best with glucose and salt.	....	Salt fish.	Förster.
Liquefying; grows best at 30° C.	....	Tropical seas.	Fischer.
Liquefying; colonies look as if punched out; grows best at 15° C.	....	Water around Kiel.	Fischer.
Movements depend upon light.	....	....	Engelman.
....	....	Stagnant water.	Ehrenberg.
Thick skin on potato.	Causes fermentation in dextrin solutions.	....	Prazmowski.
Little red colonies; liquefying rapidly; especially abundant on potatoes.	....	Bread and potatoes.	Ehrenberg.
Liquefying slowly; opaque center, irregular processes.	....	Putrefaction.	Hauser.
Liquefying quickly.	....	Putrefaction.	Hauser.
Not liquefying; thick white layer on potato.	....	Putrefaction.	Hauser.
Grows at ordinary temperature, rapidly forming on surface a brownish growth; pin-head colonies raised above surface; not liquefying.	Not virulent.	In diphtheric membrane and normal pharynx.	Wellenhof. (Hoffman.)
....	Decomposes albumin.	Human feces.	Bienstock.
On agar, a glassy growth.	....	Closed abscesses.	Rosenbach.
Liquefying; growth rapid; colonies like molds, from center radiating in all directions and through the gelatin; the air must be excluded.	Not pathogenic.	In serum of white mice inoculated with earth.	Lüderitz.

NON-PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
RADIATUS.	Streptococcus.	Small cocci in chains.	....
RAMOSUS LIQUEFACIENS.	Bacillus.	Motile rods.	....
REITENBACHII.	Merismopedia.	Cocci in packets or plates; colorless cell-wall containing chlorophyll.	....
ROSACEUS.	Micrococcus.	Large cocci in pairs and tetrads.	Red pigment.
ROSEA.	Sarcina.	Spheric cocci in cubical packets.	....
ROSEA PERSEINA.	Beggiatoa.	Long rods with cocci-shaped bodies in them, containing sulphur and a red pigment.	Pigment called bacteriopurpurin.
ROSEUM.	Spirillum.	Very short curved rods; motile and spores.	Pigment soluble in alcohol.
RUBER.	Bacillus.	Motile rods in groups.	Brick-red pigment.
RUBRUM.	Spirillum.	Motile; short spirilla; aërobic.	Pale-rose pigment.
RUFUM.	Spirillum.	Long motile spirals.	Red-rose pigment.
RUGULA.	Spirillum (vibrio).	Motile rods, in long spirals, singly and in chains, with flagella and spores; anaërobic.	....
SAPROGENES.	Bacillus.	Large rods, terminal spores; facultatively anaërobic.	....
SCABER.	Bacillus (tyrothrix).	Short motile rods in chains; spores; aërobic.	Tyrosin and leucin are formed.
SCHEURLEN'S.	Bacillus.	Short motile rods; spores.	....
SEPTICUS.	Bacillus.	Non-motile rods in threads and spores; anaërobic.	....
SERPENS.	Spirillum.	Long, lively threads, with three windings.	....
SIMILIS.	Bacillus.	Immotile rods; transparent spores.	....
SPINOSUS.	Bacillus.	Large motile rods; spores; true anaërobin.	....
SUBFLAVUS.	Micrococcus.	Diplococci colored by Gram's fluid.	....
SUBTILIFORMIS.	Bacillus.	Immotile rods in threads; transparent spores.	....

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Liquefying; white colonies with greenish tinge; funnel-shaped in test-tube.	....	Air.	Flügge.
Liquefying; concentric colonies; funnel-shaped in test-tube.	....	Air.	Flügge.
....	....	....	Caspary.
Not liquefying; small red knobs, with fecal odor.	....	Air.	Flügge.
....	....	Marshes.	Schröter.
....	....	Marshes.	Zopf.
Not liquefying; thick violet colonies; deep red on potato.	....	Blennorrhagic pus.	Mace.
....	....	Boiled rice.	Frank.
Not liquefying; grows slowly; pale-rose colonies.	....	Dead mice.	Esmarch.
....	....	Stagnant water.	Perty.
Liquefying rapidly; round yellow dots with zone; fecal odor.	Causes cellulose to ferment.	Vegetable infusions and tartar of teeth.	Müller.
Grows slowly; foul odor.	....	Putrefaction.	Rosenbach.
....	....	....	Duclaux.
Growth best at 39° C.; slowly liquefying on potato; a yellow wrinkled skin, underneath which a red color.	....	In carcinomatous and normal mamma.	Scheurlen.
....	....	Putrid blood.	Klein.
....	....	Stagnant water.	Müller.
Grows rapidly.	....	Human feces.	Bienstock.
Liquefying; spiny periphery; foul odor due to methylmercaptan.	Albuminous decomposition.	Garden-earth.	Lüderitz.
Liquefying; yellow dots.	....	Vaginal secretion and lochial discharges.	Bumm.
Grows best at 37° C.	....	Human feces.	Bienstock.

NON-PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
SUBTILIS (hay bacillus).	Bacillus.	Large motile rods, three times longer than broad, in threads, with flagella and spores; aërobic.	....
SYNCYANEUS.	Bacillus.	Same as *Cyanogenus*.	
SYNXANTHUS (yellow milk).	Bacillus.	Short, thin motile rods.	Yellow pigment, soluble in water; similar to anilin colors.
TENUE.	Spirillum.	Large motile spirals with flagella.	....
TENUIS.	Bacillus (tyrothrix).	Motile rods in long chains; spores.	....
TERMO.	Bacillus.	Short motile, cocci-like rods in zoöglea.	....
TUMESCENS.	Bacillus.	Short rods with spores.	....
TURGIDUS.	Bacillus (tyrothrix).	Short immotile rods in long chains; spores; aërobic.	Carbonate of ammonium.
ULNA.	Bacillus.	Very large rods in chains and singly; not very motile; large spores.	....
UNDULA.	Spirillum.	Long motile spirals, with flagella.	....
UREÆ.	Bacillus.	Short rods; spores; aërobic.	Ferment, propylamin.
URINÆ.	Sarcina.	Small cocci in families.	....
UROCEPHALUS.	Bacillus (tyrothrix).	Cylindric motile rods with spores; anaërobic.	....
VENTRICULA.	Sarcina.	Cubic packets of 8 to 64 cocci.	....
VENTRICULI.	Bacillus.	Rods motile, often in bundles of four.	....
VERSICOLOR.	Micrococcus.	Small cocci.	....
VIOLACEUS.	Bacillus.	Motile rods, round end; spores.	Violet pigment, soluble in alcohol.
VIOLACEUS.	Bacillus.	Immotile rods, forming large spores.	Violet pigment, like anilin.
VIRENS.	Bacillus.	Straight rods; spores; immotile; green tinged.	Supposed to contain chlorophyll.

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Liquefying; gray center, wreath-like border; thick layer on potato.	....	Soil and dust, hay, etc.	Ehrenberg.
In boiled milk a yellow pigment is formed.	....	Boiled milk and potatoes.	Ehrenberg.
....	....	Stagnant water.	Ehrenberg.
....	Precipitates casein; forms a pellicle on milk.	Fermenting cheese and milk.	Duclaux.
Liquefying; opaque center, yellow layer next, and the periphery lobed; funnel-shaped in test-tube.	....	Connected with putrefaction of plants.	Dujardin.
On boiled carrots a wrinkled, gelatinous disk.	....	Boiled carrots.	Zopf.
A pellicle formed on surface of milk; a heavy precipitate beneath.	....	Fermenting milk and cheese.	Duclaux.
On boiled egg little zoöglea.	....	Putrefying water and boiled eggs.	Cohn.
....	....	Vegetable infusions.	Müller.
Resembling a globule of fat; grows well in mucous urine.	Splits urea into ammonii carbonas.	Stale urine.	Miquel.
....	....	Bladder.	Welcker.
....	....	Fermenting milk	Duclaux.
Not liquefying.	....	Contents of stomach.	Goodsir.
Round colonies with dark center; slow growth; not liquefying.	Peptonizes albumin.	Stomach of dogs fed on meat.	Raczynssky.
Not liquefying; iridescent yellow surface.	....	Air.	Flügge.
Not liquefying; center deep violet; color remains on agar a long time.	....	Water.	Zopf.
Liquefying; transparent colonies, surrounded by violet zone.	....	Boiled potato and water.	Schröter.
....	....	Stagnant water.	VanTiegham

NON-PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
VIRESCENS.	Bacillus.	Short motile rods with flagella very broad.	Deep-green pigment, turning yellow-brown.
VIRGULA.	Bacillus (tyrothrix).	Slender immotile rods; spores aërobic.	....
VIRIDIS.	Bacillus.	Little immotile rods; oval spore, which is tinged green.	....
VISCOSUS.	Bacillus.	Motile rods, rounded ends, usually in pairs.	Green pigment.
VISCOSUS.	Micrococcus.	Streptococci of globular cells.	Gummy substance called viscosa, and ferment.
VITICULOSUS.	Micrococcus.	Oval cocci in large groups.	....
VOLUTANS.	Spirillum.	Long spirals with flagella.	....
ZOPFII.	Bacillus.	Long motile rods, breaking up into spores like cocci.	....

PART II.—

NAME.	GENUS.	BIOLOGY.	PRODUCT.
AËROGENES CAPSULATUS.	Bacillus.	Usually found in pairs, resembling diplococci; capsulated; obligate anaërobe.	Gas with characteristic odor.
ALKALIGENES.	Bacillus.	Rods like colon and typhoid, motile.	Produces alkali in mannite and milk.
ALVEI.	Bacillus.	Rods with large spores.	....
AMYLOVORUS.	Micrococcus.	Oval cells, never in chains.	Forms butyric acid.
ANTHRACIS SYMPTOMATICI.	Bacillus.	Large slender rods with swellings at spore; anaërobic.	Rancid odor.
ANTHRACIS.	Bacillus.	Straight rods, slightly concave ends; immotile; aërobic; spores.	Toxalbumin.
ARTICULORUM (diphtheriticus).	Micrococcus.	Oval cocci in long chains, identical with pyogenes.	....

BACTERIA.—(*Concluded.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Deep round colonies, the vicinity colored green; grows on surface; slow growth; not liquefying.	....	Green sputum.	Frick.
....	....	Milk.	Duclaux.
....	....	Water.	VanTiegham.
Rapid growth, liquefying; small hair-like processes from colonies; later on, viscid and in threads, with green fluorescence.	....	Water and earth.	Frankland.
....	Mucoid fermentation in wine and beer.	Beer and wine.	Pasteur.
Not liquefying; a fine network in the colony; mucoid layer on potato.	....	Air.	Flügge.
....	....	Marshes.	Ehrenberg.
Not liquefying; forms thick coils like braided hair.	....	Intestinal contents of fowls.	Kurth.

PATHOGENIC BACTERIA.

CULTURE CHARACTERS	ACTIONS.	HABITAT.	DISCOVERER.
Acid reaction in litmus milk; coagulates casein with cavity-formation due to gas.	Causes fermentation; can produce gas from proteid alone.	Intestinal contents; earth; water; raw foods.	Welch.
Colonies like typhoid.	Feces and water.	....	Petruschky.
Liquefying; growths radiating from center downward; on potato a dry yellow layer.	Produces a disease in bees called "foul brood."	Larvæ of bees.	Cheshire and Cheyne.
....	"Fire-blight" in pear trees.	....	Burrill.
Liquefy gelatin; grow only in atmosphere of hydrogen.	Causes quarter evil in animals.	Blood and tissues.	Bollinger.
Liquefying; granular colonies with irregular border; on potato a dry, creamy layer; in test-tube a thorny, prickly track.	Causes splenic fever in animals; malignant pustule in man.	Found in tissues and excreta of diseased animals.	Rayer and Davaine.
Grows well on gelatin; pale-gray colonies; not liquefying; slow growth on potato.	Fatal in mice and rabbits.	Mucous membrane of diphtheria.	Löffler and Cohn.

PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
AVISEPTICUS. See	*Hemorrhagic Se*	*pticemia.*	
BOMBYCIS.	Micrococcus.	Oval cocci in chains and zoöglea; motile.	....
BORDET-GENGOU.	See *Whooping-*	*cough.*	
BOTULINUS.	Bacillus.	Large rounded ends; motile; flagellated; anaërobic.	Butyric acid; and a powerful toxin.
BOVISEPTICUS. See	*Hemorrhagic Se*	*pticemia.*	
BUBONIC PLAGUE (Pestis).	Bacillus.	Short thick rods with indistinct capsule.	....
BUCCALIS.	Leptothrix.	Long threads in thick bundles, containing masses of cocci and spirals.	....
CATARRHALIS.	Micrococcus.	Diplococci at times resembling gonococcus.	....
CATTLE PLAGUE (Texas fever).	....	See *Hemorrhagic Sep*	*ticemia* and *Swine*
CAVICIDA.	Bacillus.	Little rods twice as long as broad.	Propionic acid through decomposition of sugar.
CHAUVÆI. See	*Symptomatic*	*Anthrax* (Rauschbra	nd).
CHOLERA ASIATICÆ.	Spirillum.	Motile, spiral-shaped rods, often in chains; very short flagella on ends, and strictly aërobic; spores have not been found.	Ptomaïn-like muscarin and toxalbumin, soluble in water.
CHOLERÆ GALLINARUM (chicken cholera).	Bacillus.	Immotile, cocci-like rods; without spores; strictly aërobic.	Toxalbumin.
CHOLERA NOSTRAS (Finckler).	Spirillum.	Motile, comma-shaped rods; strictly aërobic.	....
COLI COMMUNIS.	Bacillus.	Short motile rods, slightly curved, without spores; facultatively anaërobic.	....
CRASSUS SPUTIGENUS.	Bacillus.	Short, thick rods with rounded ends.	....
DECALVANS.	Micrococcus.	Spheric cells in great numbers.	....

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
....	Causes "flacherie" in silkworms.	Intestines of silkworms.	Béchamp.
Gelatin colonies appear as small semi-transparent spheres.	Sausage and meat poisoning.	Intestine of pig.	Van Ermengem.
Does not liquefy gelatin; white, point-like colonies turning gray and then brown.	Causes bubonic plague	Tissues, body fluids, and secretions of plague patients.	Versin and Kitasato.
....	Causes dental caries.	Teeth slime.	Robin.
At 37° on agar, round, gray colonies, serrated edges. *Plague.*	Not pathogenic.	Mucous secretions healthy persons.	R. Pfeiffer.
Not liquefying; irregular scale-like colonies, making the gelatin viscid.	Kills guinea-pigs.	Human feces.	Brieger.
Liquefying slowly, small depressed scars giving a frosted appearance, or like ground glass; on potato, a thin brown layer; in test-tube, a funnel-shaped liquefaction, with a bubble of air in the top, the funnel taking six or seven days to form well.	Causes cholera Asiatica in man and a similar trouble in animals.	Feces of cholera patients.	Koch.
Not liquefying; small isolated white disks; in test-tube, a granular track; very faint.	Causes chicken cholera in fowls; not acting on man.	Blood and feces of diseased fowls.	Pasteur.
Liquefying rapidly; colonies yellow-brown thick masses; in test-tube, funnel formed in twenty-four hours, dissolving all gelatin in two days; profuse gray mass on potato.	Harmless in man; fatal to guinea-pigs.	Feces of cholera nostras and caries of teeth.	Finckler and Prior.
Not liquefying; dark center, undulated periphery; green-colored layer on potato; milky layer on surface of test-tube.	Fatal to guinea-pigs and rabbits; causes diarrhea in man; ferments sugar.	Feces of nursing infants; water; choleraic stools.	Escherich.
Not liquefying;, oval, grayish, slimy colonies; nail-shaped growth in test-tube.	Mice and rabbits die in forty-eight hours with gastro-enteritis.	Sputum.	Kreibohm.
....	Causes alopecia areata.	In roots of hair.	Thin.

PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
DENTALIS VIRIDANS.	Bacillus.	Slightly curved rods, round ends.	Gray pigment.
DIARRHEA OF INFANTS.	Bacillus.	Motile, medium-sized rods; spores; aërobic.	Toxalbumin.
DIARRHEA OF MEAT-POISONING (*Enteritidis sporogenes*).	Bacillus.	Rods in groups of two and singly; round ends; spores.	....
DIPHTHERIÆ.	Bacillus.	Immotile, middle-sized rods, rounded ends; facultative anaërobic.	Toxalbumin.
DIPHTHERIA OF CALVES (Vitulorum).	Bacillus.	Long rods in threads.	....
DIPHTHERIA IN PIGEONS (Columbarum).	Bacillus.	Short rods in groups.	....
DIPLOBACILLUS OF CONJUNCTIVITIS.	Bacillus.	Non-motile; usually occurs in pairs.	....
DUCK CHOLERA.	Bacillus.	Similar to chicken cholera bacillus; immotile.	....
DYSENTERIÆ.	Bacillus.	Resembles typhoid bacillus.	First slightly acid, then alkaline.
DYSENTERY (epidemic).	Bacillus.	Short motile rods; very thin.	....
ENTERITIDIS.	Bacillus.	Resembles typhoid bacillus.	....
ENTERITIDIS SPOROGENES.	See *Diarrhea of Meat Poisoning.*		
ERYSIPELAS OF SWINE (Rothlauf; rouget du porc).	Bacillus.	Small, slender motile rods; facultatively anaërobic.	Two vaccines, which give immunity.
FŒTIDUS OZÆNÆ.	Bacillus.	Short rods, very motile; in pairs and chains.	Foul gas.
FROG PLAGUE.	Bacillus.	See *Swine Plague.*	
GANGRENE.	Micrococcus.	Oval cocci in zoöglea.	....
GIGANTEA.	Leptothrix.	Long rods, cocci and short rods in one; thread also spiral.	....

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Not liquefying; round, sharply outlined colonies, with bluish gray opalescence.	Septic processes and death in mice and pigs.	In caries of teeth.	Miller.
Not liquefying; green colonies with foul odor.	Causes green diarrhea in animals when intravenously injected, and is the cause of green diarrhea in infants.	Feces of infants suffering from green diarrhea.	Lesage.
....	Causes death in animals, with symptoms of septicemia.	Blood and juices of choleraic diarrhea.	Klein.
Not liquefying; little yellowish colonies; a membranous layer on potato.	Gives rise to diphtheria in man and animals.	Diphtheric exudate.	Löffler (Klebs).
....	When inoculated in mice causes death.	Diphtheric membrane of calf.	Löffler.
Whitish patches.	Necrosis in pigeons and other animals.	Diphtheric membrane in pigeons.	Löffler.
Addition of blood-serum to media necessary; liquefying.	Found in subacute conjunctivitis.	Conjunctival secretion.	Morax.
Small round yellow colonies like wax-drops; not liquefying.	Fatal for ducks, but not for chickens or pigeons; less active than chicken cholera; causes diarrhea and exhaustion.	Blood of diseased ducks.	Cornil and Toupet.
Resembles typhoid bacillus in many respects.	Produces one variety of dysentery.	In dysenteric stools.	Shiga.
Not liquefying; concentrically arranged colonies; dry yellow membrane on potato.	The cause of epidemic dysentery in man; enteritis in guinea-pigs.	In feces and mesenteric glands.	Chantemesse and Widal.
Resembles typhoid except that it ferments dextrose.	Produces enteritis in man and animals.	Intestinal contents; its toxin in meat of diseased animals.	Gärtner.
Very delicate silver-gray clouds on the gelatin, like bone-cells; not liquefying; in test-tube a very faint clouding.	Causes erysipelas in swine and other animals; the German "Rotlauf," French "rouget du porc."	Blood and organs of diseased animals.	Löffler.
Small greenish colonies which soon become liquefied and indistinguishable; a foul odor produced.	Mice are killed by injection; rabbits affected with progressive gangrene.	Secretion of persons suffering from ozena.	Hajek.
Grayish colonies with foul odor.	....	Gangrenous tissue.	Eberth.
....	Causes caries of teeth.	Diseased teeth of animals.	Miller.

PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
GINGIVÆ PYOGENES.	Bacillus.	Short thick rods with rounded ends.	....
GLANDERS (Rotz, Mallei).	Bacillus.	Slender, immotile rods; usually singly; spores; facultatively anaërobic.	....
GONORRHŒÆ (Gonococcus).	Micrococcus.	Diplococci kidney-shaped; motile; do not color with Gram.	....
GROUSE DISEASE.	Bacillus.	Small rods and oval cocci in chains; immotile.	....
HÆMATOCOCCUS BOVIS.	Diplococcus.	Cocci seldom in chains; surrounded by a pale zone.	....
HÆMOPHILIA NEONATORUM.	Micrococcus.	....	....
HEMORRHAGIC SEPTICEMIA (Infectious Pleuropneumonia, Wild Plague, German Swine Plague, Cattle Plague, Steer Plague, Rabbit Septicemia).	Bacillus.	Short rods, twice as long as broad; immotile.	....
HOG CHOLERA (Swedish swine plague).	Bacillus.	Very motile oval rods, similar to hemorrhagic septicemia.	Peptonizes milk without coagulation.
ICTEROIDES.	Bacterium.	Once supposed to be the cause of yellow fever. Identicalwth *Sanarelli*.	....
INFLUENZA.	Bacillus.	Very minute rods or in clumps.	....
INSECTORUM.	Micrococcus.	Oval cells in chains and zoöglea; streptococci.	....
INTRACELLULARIS MENINGITIDIS.	Diplococcus.	Resembles gonococcus in morphology and arrangement in interior of leukocytes.	....
KOCH-WEEKS.	Bacillus.	Resembles influenza bacillus.	....
LACTIS AËROGENES.	Bacillus.	Short, thick immotile rods.	....

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Growth rapid; liquefying; round colonies, visible to naked eye in twenty-four hours.	Fatal to mice, with septic processes.	Suppurating pulp of tooth.	Miller.
Light yellow, like honey, colonies, turning red-brown in a few days.	Glanders is caused by the bacillus in man and animals.	In epithelium and ulcerated glands.	Löffler.
Grow on blood-serum.	Gonorrhea in man.	Gonorrheal pus; in pus-cells and epithelium.	Neisser.
Not liquefying; small scales which turn gray in a few days, the edges serrated.	Fatal for mice and guinea-pigs.	In blood and organs of diseased grouse.	Klein.
Best at 38° C.; not liquefying; small white points; sparse growth on potato; transparent.	Fatal for rabbits and rats; hyperemia of lungs and spleen; blood - exudate in peritoneal cavity.	Blood and organs of animals diseased with hemoglobinuria.	Babes.
....	Supposed to be the cause of the disease.	Found in this disease.	Klebs.
White isolated pinhead points, not growing on potato; best at 37° C., not liquefying.	A disease having different names in different animals, characterized by edema, hemorrhage, and septicemia.	Blood and serum of diseased animals.	Hüppe.
Very good growth on gelatin and potatoes; a yellow-brown color.	In experiment, animal's death in four to eight days; bacteria in little emboli in capillaries.	Not spread through tissue, but in capillaries of diseased swine.	Salmon and Selander.
....	....	....	....
Grow best on blood-agar; colonies very small, almost transparent.	Produces epidemic influenza.	Secretions of respiratory tract.	Pfeiffer, Kitasato, Canon.
....	A contagious disease in the chinch-bug.	Stomach of chinch-bug.	Burrill.
Transparent colonies, forming thin layer on Löffler's blood-serum and glycerin agar.	Causes epidemic cerebrospinal meningitis.	In cerebrospinal fluid and nasal secretions.	Weichselbaum.
Rarely grows, except on serum agar.	Most common cause of acute contagious conjunctivitis.	Conjunctival secretion.	Koch and Weeks.
Small, porcelain-like disks with depressed center; funnel-shaped in test-tube with gas.	Fatal to guinea-pigs and rabbits; coagulates milk; decomposes sugary solutions.	Feces of nursing infants and of cholerine.	Escherich.

PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
LEPRÆ.	Bacillus.	Slender, immotile rods with pointed ends.	....
LIQUEFACIENS CONJUNCTIVÆ.	Micrococcus.	Single cocci; never in threads.	....
LUPUS.	Bacillus.	Same as *Tuberculosis.*	
MALIGNANT EDEMA (Gangrenous Septicemia, Vibrio Septique).	Bacillus.	Large, slender rods, rounded ends, often in threads; motile, with flagella and spores; strongly anaërobic.	Soluble vaccine.
MAMMITIS OF COWS.	Micrococcus.	Oval cocci in chains; streptococci; facultatively anaërobic.	....
MAMMITIS OF SHEEP.	Micrococcus.	Streptococci and in fours.	....
MELITENSIS (Malta Fever).	Micrococcus.	5μ in diameter; occurs singly or in chains of two or more; said to be flagellated.	....
METCHNIKOVI	Spirillum (vibrio).	Motile spirals with flagella; aërobic.	An alkaline vaccine which will cause immunity.
NEAPOLITANUS.	Bacillus.	Small immotile rods, with rounded ends; no spores; facultatively anaërobic.	Produces acids in gelatin cultures.
NOMÆ.	Bacillus.	Small rods, with rounded ends, growing often in long threads.	....
OLEÆ.	Bacillus.	Motile aërobic; does not liquefy gelatin.	Alkali in milk.
OXYTOCUS PERNICIOSUS.	Bacillus.	Short rods with round ends.	....
PARATYPHOID.	Bacillus.	Resembles typhoid bacillus.	Indol sometimes produced.
PERFRINGENS. See	*Aërogenes capsulatus.*		
PESTIS. See *Bubonic Plague.*			

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
On blood-serum round white plaques with irregular borders.	Causes leprosy in man and animals.	Leprous tissue.	Hansen.
Liquefying; growth rapid; colonies on surface, with little radiating branches from a dark center; those in deep, berry-shaped.	On cornea of rabbits causes slight clouding.	Normal human conjunctiva.	Gombert.
Liquefying; thick center, radiating periphery; in high culture in test-tube, gas-bubbles arise, with foul odor.	Animals quickly die with extensive gangrene and edema.	Garden-earth.	Pasteur.
Not liquefying; brown, round granular colonies; grows slowly; in test-tube, heavy deposit along the needle's track.	Causes contagious mammitis in cows: coagulates milk.	Mammary gland.	Nocard and Mollereau.
Liquefying; round centers with zone of liquefaction; cone-shaped in test-tube.	Causes contagious gangrenous mammitis in sheep.	Found in the milk of diseased sheep.	Nocard.
Small, round, slightly raised disks; do not liquefy.	Causes Malta fever.	Best obtained from spleen.	Bruce.
Grows quickly; colonies, some like cholera Asiatica, others like cholera nostras; liquefying.	Causes vibrion septicemia in guinea-pigs and pigeons.	Feces of fowls.	Gamaleia.
Not liquefying; thin pearl like scales in several layers; wrinkled and mucous layers on potato.	Causes death in some animals; not the cause of cholera.	Cholera epidemic of Naples, 1884.	Emmerich.
Granular spheric colonies in the deep, flat on the surface; not liquefying; growth rapid; best at 35° C.	No action on mice or rabbits.	In necrotic tissue of noma.	Schimmelbusch.
Whitish growth on culture-media.	Causes olive-gall on olive plant.	....	Savastano.
Small yellow granular colonies; nail-culture in test-tube.	Intravenous injection causes death in mice and rabbits; turns milk acid.	Sour milk.	Wyssokowitsch.
Ferments glucose, but not lactose or saccharose; does not coagulate milk.	Causes continued fevers.	Intestinal contents.	Widal, Gwyn, Schottmüller.

PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
PNEUMONIA (Pneumococcus of Friedländer).	Bacillus.	Short, immotile rods, singly or in diplococci, surrounded with capsule; no spores; not colored with Gram; facultatively anaërobic.	....
PNEUMONIA (Pneumococcus of Fränkel; Micrococcus of Pasteur).	Bacillus.	Short, oval rods, often in chains; immotile; no spores; in the tissue surrounded with capsule, colored with Gram; facultatively anaërobic.	....
PNEUMONICIS AGILIS.	Bacillus.	Short, thick motile rods in pairs.	....
PROTEUS SEPTICUS.	Bacillus.	Slightly curved rods, swelled in portions, sometimes in long threads; motile.	Foul gas.
PSITTACI (perniciosus).	Micrococcus.	Streptococci and zoöglea.	....
PYOCYANEUS.	Bacillus.	Thin, motile rods; facultatively anaërobic.	Pyocyanin, a non-poisonous pigment.
PYOCYANEUS β.	Bacillus.	Forms a brown-yellow pigment; otherwise identical with above.	....
PYOGENES (Streptococcus erysipelatis—Fehleisen).	Micrococcus.	Streptococci and zoöglea.	....
PYOGENES ALBUS.	Micrococcus.	Staphylococci and streptococci; facultatively anaërobic.	....
PYOGENES AUREUS (micrococcus of osteomyelitis, Becker).	Micrococcus.	Staphylococci and zoöglea; facultatively anaërobic.	Ptomaln, toxalbumin, and pigment.
PYOGENES CITREUS.	Micrococcus.	Same as *Pyogenes aureus.*	....
PYOGENES FŒTIDUS.	Bacillus.	Short motile rods in pairs.	....
PYOGENES TENUIS.	Micrococcus.	Cocci without definite arrangement.	....
RELAPSING FEVER (Obermeier).	Spirillum.	Long, wavy spirals; motile.	....

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Does not liquefy; grows quickly; a button-like colony; in test-tube, as if a nail driven in the gelatin with head on surface.	An accompaniment of pneumonia, not a cause; animals not affected.	Pneumonic and other sputum, and lung tissue.	Friedländer.
Does not liquefy; grows slowly; small, well-defined masses; in test-tube, little separate globules, one above the other.	Causes pneumonia in man, septicemia in animals; also serous inflammations in man, as pleurisy, peritonitis, etc.	Sputum of lung affections and serous inflammations.	A. Fränkel.
Liquefying; dark granular colonies; thick sediment in test-tube.	Pneumonia in rabbits.	From rabbits' pneumonia.	Schön.
Growth rapid; liquefying; colonies have foul odor, are small, thick branches, but soon all liquid.	Fatal for mice in one to three days.	From a child dying of intestinal gangrene.	Babes.
....	Causes disease in gray parrots.	In blood of parrot's disease.	Wolff.
Liquefying; large, flat colonies with greenish fluorescence; on potato, yellow-green skin; deeply coloring the pulp.	Fatal for animals; colors the dressings green.	Pus.	Gessard.
....	....	....	Ernst.
Not liquefying; round punctiform colonies; slow-growing.	Suppuration and septicemia in animals.	Pus.	Rosenbach.
Liquefying; white opaque colonies.	Suppuration and abscess.	Pus.	Rosenbach.
Liquefying; small colonies with a yellow-orange pigment in center; yeast-like smell; a moist layer on potato.	Causes abscesses and suppuration in man and animals.	Pus.	Rosenbach.
Colonies, citron-yellow color.	Suppuration.	Pus.	Passet.
Not liquefying; mucous layer on potato; very thick; in test-tube, a slight layer on surface, and small points along the track.	Fatal to animals.	Pus.	Passet.
On surface, transparent; thin growth; grows slowly.	....	Pus of abscesses.	Rosenbach.
Cannot be cultivated.	Causes fever in man and animals, and is the cause of relapsing fever.	Blood of man during an attack of the disease.	Obermeier.

PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
RHINOSCLEROMA.	Bacillus.	See *Pneumococcus* of	Friedländer, with
SALIVARIUS PYOGENES.	Micrococcus.	Very small round cocci and staphylococci.	....
SALIVARIUS SEPTICUS.	Bacillus.	Short, immotile rods, encapsulated in pairs, sometimes long chain; aërobic.	....
SALIVARIUS SEPTICUS.	Micrococcus.	Cocci singly and in zoöglea; aërobic.	....
SAPROGENES No. II.	Bacillus.	Short rods; facultatively anaërobic.	Foul gas.
SAPROGENES No. III.	Bacillus.	Very short rods; facultatively anaërobic.	Foul gas.
SAPROGENES FŒTIDUS.	Bacillus.	Immotile rods; spores.	Foul gas.
SENILE GANGRENE.	Bacilli.	Thin rods; immotile; singly and in pairs; ends somewhat thickened; aërobic; spores.	....
SEPTICEMIA AFTER ANTHRAX.	Micrococcus.	Motile streptococci.	....
SEPTICEMIA OF MICE.	Bacillus.	Smallest bacillus known; immotile.	....
SEPTICEMIA OF RABBITS (Cuniculicida).	Bacillus.	See *Hemorrhagic Septicemia.*	
SEPTICUS ACUMINATUS.	Bacillus.	Thin, lancet-shaped rods; very slender.	....
SEPTICUS AGRIGENUS.	Bacillus.	Very short rods.	....
SEPTICUS LIQUEFACIENS.	Micrococcus.	Streptococci and diplococci.	....
SEPTICUS ULCERIS.	Bacillus.	Oval rods; motile.	Gas; no odor.

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
which it is identical.	....	....	Frischl.
Slowly liquefying; small white opalescent colonies.	Local abscess in animals.	Saliva.	Biondi.
Not liquefying; gray circular colonies; transparent zone; in test-tube, separated.	Fatal to animals.	Saliva of healthy persons.	Biondi.
Not liquefying; round colonies; separated dots in test-tube.	Fatal to animals.	Saliva of puerperal women.	Biondi.
Grows quickly; on agar, hyaline drops which quickly coalesce, and form a mucoid layer with a foul odor, that of perspiring feet.	Produces septicemia in rabbits.	Sweat of feet.	Rosenbach.
Forms a fluid gray band on agar; odor of putrefaction.	Suppuration in rabbit.	Putrid marrow of bone.	Rosenbach.
Not liquefying; thin, transparent layer; putrid odor.	Rabbits killed with large doses.	Mesenteric glands of swine with erysipelas and of healthy swine.	Schottelius.
Round yellow colonies; liquefying in thirty-six hours; best growth at 37° C.	Causes gangrene in mice, similar to senile gangrene of man.	In gangrenous tissue and blood of senile gangrene.	Tricomi.
In bouillon virulence destroyed.	Septicemia in rabbits, but not in chickens or guinea-pigs.	Blood of animal dead from anthrax.	Charrin.
Not liquefying; small flocculent masses in the deep; grows very slowly; in the test-tube producing a faint cloud.	Septicemia in house-mice, but not field-mice.	Putrefying liquids.	Koch.
At 37° C. on blood-serum small transparent plates; later on, turning yellow.	Pathogenic for rabbits and guinea-pigs; fever; and bacilli in blood and organs.	Navel stump of child dead of septicemia.	Babes.
Not liquefying; brown center, a ring, then yellow zone.	Septicemia in mice and rabbits.	Earth of recently plowed fields.	Nicolaier.
Liquefying; a thin granular streak, the surface sunken in; later, cone-like, the walls covered with leaf-shaped colonies.	Pathogenic for mice and rabbits, producing edema, in the serum of which the cocci abound.	Blood and organs of child dying of septicemia.	Babes.
Liquefying; yellow colonies, taken up with gas later on.	An ulcer in inoculated animals, followed by paralysis and death.	In blood of child with gangrenous ulcer.	Babes.

PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
SEPTICUS VESICÆ.	Bacillus.	Rods always single; very motile; oval spores.	....
SMEGMA.	Bacillus.	Slender curved rods, identical with what was known as syphilis bacillus of Lustgarten.	....
SOFT CHANCRE.	Bacillus.	Minute oval rods, chiefly in groups or chains.	....
SPUTIGENUM.	Spirillum.	Curved, comma-shaped rods; motile.	....
SUBFLAVUS.	Micrococcus.	Diplococci like gonococci; colored by Gram.	....
SWINE PLAGUE (American and French).	Bacillus.	Motile, oval rods, similar to that of hog cholera.	Causes casein precipitate in milk and acid formation.
SYCOSIFERUS FŒTIDUS.	Bacillus.	Short, straight immotile rods, often in threads.	On potatoes a foul odor.
SYPHILIS (Spirocheta pallida).	Spirocheta.	Small delicate spirals, difficult to stain.	....
TETANUS.	Bacillus.	Large, slender motile rods, with spores in one end, drumstick shape, often in threads; true anaërobic.	Ptomaïns, tetanin, tetanotoxin, spasmotoxin; also a toxalbumin.
TETRAGENUS.	Micrococcus.	Large round cells, united in groups, usually of four, and surrounded by a capsule; immotile; aërobic.	....
TIMOTHY GRASS.	Bacillus.	Extremely acid-fast; resembles tubercle bacillus; in cultures may show club formation and branches	....
TOXICATUS.	Micrococcus.	Cocci singly and in pairs.	....

BACTERIA.—(*Continued.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Not liquefying; small pin-head colonies, growing slowly; never larger; a brown center, yellow periphery.	Pathogenic for mice and rabbits, producing death.	In urine of cystitis.	Clado.
Not cultivated.	....	Normal preputial secretions.	Alvarez and Tavel.
Has not been cultivated.	Produces soft chancre.	In the sore.	Ducrey.
Not cultivated.	Causes death in animals.	In caries of teeth and saliva.	Lewis.
Growth slow; liquefying; on tenth day yellow points with thready boundary; on potato, a brown, thread - like growth after two weeks.	No result on mucous membrane; injected under skin, abscess results.	Normal secretion of vagina and urethra.	Bumm.
Not liquefying; growth similar to typhoid germ; on potatoes good growth.	Found in American and French swine plague, in frog plague, and Texas fever; animals affected locally.	Found in capillaries in little emboli; not spread in organs of diseased animals.	Billings, Rietsch, and Eberth.
Slow growth; not liquefying; after four days, little white points, which do not change for several weeks, then the superficial ones are mucus-like; nail growth; on potatoes, rapid growth.	On human skin causes eruption, vesicular around hairs, then it becomes pustular; similar to sycosis.	From sycosis of the beard.	Tommasoli.
On blood-serum thin growth.	Supposed to cause syphilis.	In tissue and secretions of syphilitics.	Schaudinn.
Liquefy gelatin slowly; colonies have radiated appearance; a thorny growth along the track in test-tube.	Produces tetanus in man and animals.	Earth and manure.	Nicolaier and Kitasato.
Not liquefying; little porcelain-like disks; thick slimy layer on potato.	Fatal to guinea-pigs and white mice.	Found in cavernous phthisical lungs.	Gaffky.
Colonies visible in thirty-six hours, scale-like and grayish white.	May produce tubercles.	Infusions of timothy grass.	Moeller.
....	Supposed to be the cause of *Rhus* (poison ivy) poisoning.	Found in the *Rhus toxicodendron.*	Burrill.

PATHOGENIC

NAME.	GENUS.	BIOLOGY.	PRODUCT.
TUBERCULOSIS.	Bacillus.	Slender rods, usually in pairs; not motile; spores not definitely determined; facultatively anaërobic.	Kochin or paratolin, a glycerin extract of the pure culture (tuberculin).
TYPHOID.	Bacillus.	Slender motile rods, sometimes in threads; flagella, but no spores; facultatively anaërobic.	Typhotoxin and toxalbumin.
TYPHOID OF SWINE (swine plague).	....	See *Swine Plague.*	
TYROGENUM.	Spirillum (vibrio).	Spiral-shaped rods; aërobic.	....
WHOOPING-COUGH.	Bacillus.	Short oval.	....
WELCHII.	See *Aërogenes capsulatus.*		
XEROSIS.	Bacillus.	Similar to diphtheria bacillus.	....

BACTERIA.—(*Concluded.*)

CULTURE CHARACTERS.	ACTIONS.	HABITAT.	DISCOVERER.
Grows best on blood-serum and glycerin agar at 37° C., forming little white crumbs on the surface; under microscope a hairy, matted coil is seen; growths on potatoes when air-tight have been obtained.	Causes tuberculosis, local and general, in man and lower animals.	In all organs and secretions of tubercular persons.	Koch.
Not liquefying; little whetstone-shaped yellow colonies in the deep, and leaf-shaped ones on the surface; on potato, a very transparent, moist layer.	Gives rise to enteric or typhoid fever in man.	Found in dejecta and spleen and urine of typhoid patients.	Eberth.
Liquefy rapidly; small round colonies; dark funnel-shaped liquefaction in test-tube.	Several animals have died from inoculations.	From old cheese.	Deneke.
Blood agar. White growth on surface.	Produces spasmodic cough in animals.	Found in whooping cough.	Bordet-Gengou.
Differs from diphtheria bacillus in not producing acid in bouillon.	....	Found in pathologic conditions of conjunctiva, sometimes in normal eye.	Kuschbert and Neisser.

INDEX

ABBÉ'S condenser, 44
Achorion schönleinii, 224
Acid, boric, 264
  curd cheese, 254
  dyes, 48
  salicylic, 264
Acid-fast bacteria, 116
Actinomyces, cladothrix, 228
  streptothrix, 228
Actinomycosis, 228
Aërobes, 28
  facultative, 28
  obligate, 28
Aërobioscope, 234
African tick fever, 218
Agar, bile salt, MacConkey's, 75
  blood-, 76
  fuchsin-lactose, 77
  glycerin-, 69
  lactose litmus, 72
  nutrient, 71
Agar-agar, 69
Age, influence of, on bacteria, 30
Agglutination test for tubercle bacilli, 121
  in cholera, 152
  in typhoid fever, 140
  Pfeiffer's cholera, 153, 154
Agglutinins, 40
Aggressins, 32
Air, bacteria in, 232
  Hesse's method of collecting, 233
  Petri method of collecting, 232, 233
  Sedgwick-Tucker method of collecting, 234
  varieties, 234
  examination of, 232
  sewer, bacteria in, 235
Albumin, blood, solution dried, 78
Alcohol, 264
  fermentation, 255
Alcoholic solution, saturated, 49
Alexin, 40
Algæ, fission, 21
Alkaline anilin-water solutions, 51
  methylene-blue, 50
  stains, 49
Alkaloids, cadaveric, 34
Allergy, 42
Altered reactivity, 42
Alum, 236
Amboceptors, 40, 42
Amebæ, examination for, 199
Ammonia production, 245
Amœba coli, 198
  dysenteriæ, 198
Anaërobes, 28
  facultative, 28
  obligate, 28
  true, 28
Anaërobic bacteria, 180
  cultivation, 82
    Botkin's method, 84
    Buchner's method, 84
    Esmarch's method, 83
    Fränkel's method, 84
    Hesse's method, 83
    Liborius's method, 82
    Park's method, 85
    Roux's method, 84
    Wright's method, 85
Anaphylaxis, 42
Anilin dyes, 47, 264
  acid, 48
  basic, 48
Anilin-oil, 48
  water, 49, 50
  dyes, 50

Animal inoculation, 91
celloidin sacs, 93
cerebral membranes, 93
cutaneous, 91
eye, 92
guinea-pigs, 91
intraduodenal, 93
intraperitoneal, 92
intratracheal, 93
intravenous, 92
methods, 91
obtaining material after, 94
subcutaneous, 92
Animals as culture-media, 81
tuberculosis in, 119
Anthrax 105
symptomatic, 187
Anthraxin, 109
Antiformin method of collecting tubercle bacilli, 117
Antigens, 41
Antisepsis, 261, 262
Antiseptics, 261, 262
Antistreptococcic bacterins, 166
serum, 166
vaccines, 166
Antitoxic serum, 39
unit, 133
Antitoxin, formation of, according to lateral-chain theory, 37
of diphtheria, 131
specific action, 38
tetanus, 183
unit for, 183
Antituberculous serum, 122
Antityphoid bacterins, 139
vaccines, 139
Arnold's steam sterilizer, 64
Artesian well water, 236
Arthrosporous bacteria, 26
Artificial cultivation, 62
Asbestos, 236
Asexual cycle in man, 199
Aspergillus flavus, 226
fumigatus, 226
glaucus, 226
Asporogenic bacteria, 26
Autoclave, 64, 65
Avicidus bacillus, 193
Azofication, 245

Bacillary dysentery, 199
Bacillus acidi lactici, 249
aërogenes capsulatus, 186
amylobacter, 251
amylovorus, 232
anthracis, 105
avicidus, 193
avisepticus, 192
Boas-Oppler, 101
botulinus, 144
bovisepticus, 192
butyricus, 251
capsule, of Pfeiffer, 159
chauvei, 187
coli, 134
diagnostic points of, 241
in milk, Wisconsin test for, 255
in urine, 102
in water, examination for, 240
colon, 134
bacillus typhosus and, differentiation, 142
comma, of cholera, 148
cyanogenes, 251
diphtheria, 126
products of, 131
dysenteriæ, 145, 199
products, 147
enteritidis, 135
sporogenes, 187
fluorescens, 171
hay, 100
icteroides, 219
Klebs-Löffler 126,
Koch-Weeks, 161
lactis aërogenes, 249
lepra, 122
mallei, 124
megaterium, 99
melitensis, 162
mesentericus vulgatus, 98
Milzbrand, 105
murisepticus, 195
mycoides, 99
œdematis, 184
maligni, 184
of anthrax, 105
of bluish-green pus, 171
of bubonic plague, 190
of chancroid, 178

Bacillus of chicken cholera, 193
of cholera, 148
of diphtheria, 126
products, 131
of dysentery, 145
products, 146
of enteric fever, 135
of erysipelas of swine, 194
of fowl septicemia, 193
of glanders, 124
of influenza, 160
of lepra, 122
of malignant pustule, 105
of pertussis, 161
of phlegmonous emphysema, 186
of rhinoscleroma, 159
of soft chancre, 178
of splenic fever, 105
of symptomatic anthrax, 187
of tetanus, 180
of typhoid fever, 135
paracolon, 143
paratyphoid, 143
perfringens, 187
pestis, 190
pneumoniæ, 159
potato, of Flügge, 98
proteus vulgaris, 147
pyocyaneus, 171
ramosus, 99
root, 99
rotz-, 124
saccharobutyricus, 187
Shiga, 199
smegma, 123
subtilis, 100
suipestifer, 133
suisepticus, 192
tubercle, 110
products of, 120
tuberculosis, 110
products of, 120
typhosus, 135
colon bacillus and, differentiation, 142
from blood, 143
in water, 138
diagnostic points, 242
products, 139
violaceus, 102
Bacillus vulgatus, 98
Welchii, 186
Wurzel, 99
X, 219
Bacteremia, 33
Bacteria, acid-fast, 116
aërobic, 28
anaërobic, 28, 180
cultivation of, 82
Botkin's method, 84
Buchner's method, 84
Esmarch's method, 83
Fränkel's method, 84
Hesse's method, 83
Liborius's method, 82
Park's method, 85
Roux's method, 84
Wright's method, 85
and soil fertility, 244
arthrosporous, 26
asporogenic, 26
avenues of entrance, 32
biologic activities, 26, 28
Brownian movements, 23
causing disease, 29
chemic activities, 26
composition, 22
products, 29
chief characteristics, 268–303
cholera, 148
classification, 22
colonies of, growth and appearances, 87
colon-typhoid group, 133
cultivation, 62
defenses to invasion, 34
development of, 21
diseases from, 29
distribution, 26
effects of, general, 33
local, 33
endosporous, 24
facultative, 27
fermentation by, 29
filterable, 218
fluorescence of, 30
gas-forming, 30
general effects, 33
growth of, 27
how cause disease, 30
in air, 232

Bacteria in air, Hesse's method of collecting, 233
Petri method of collecting, 232, 233
Sedgwick-Tucker method of collecting, 234
varieties, 234
in artesian well water. 236
in blood, 260
in butter, 254
in buttermilk, 255
in cheese, 254
in condensed milk, 255
in conjunctiva, 258
in cream, 248
in ear, 259
in feces, 260
in filtered water, 236
in food, 246
in genito-urinary passages, 260
in ice-cream, 248
in intestine, 259
in milk, 246, 249
in mouth, 258
in nasal cavity, 259
in pneumonia, 155
in sewer air, 235
in skin, 257
in soil, 232, 243
in stomach, 259
in urethra, 260
in urine, 102, 260
in vagina, 260
in water, method of examination, 238
varieties, 238
in well water, 236
infective, 33
influence of age on, 30
of electricity on, 28
of heat on, 28
of light on, 28
of moisture on, 28
of oxygen on life and growth of, 27
of Röntgen rays on, 28
of temperature on life and growth, 27
local effects, 33
locomotion, 23
methods of studying, 43
Bacteria, nitrification by, 29
non-pathogenic, 29
table of, 268–287
odors from, 30
of hemorrhagic septicemia, 192
origin, 26
oxidation by, 29
parasitic, 27
pathogenic, 29, 97
table of, 287–303
phosphorescence by, 30
pigmentation by, 30
plant diseases due to, 231
pyogenic, 33
quantity of, infection depending on, 32
reduction by, 29
reproduction, 24
sewage, examination for, 240
specific, 33
nature, 27
spore contents, 25
formations, 24
staining of, 47
structure of, 21, 22
suppurative, 33
tables of, 268–303
types, 21
ultra-microscopic, 218
unstained, examination of, 45
vibratory movements, 23
Bacterial vaccines, 95
treatment of sewage, 243
Bactericides, 41
Bactericie du charbon, 105
Bacterins, 95
anticholera, 153
antistreptococcic, 166
antityphoid, 139
counting of, 96
preparation of, 95
standardization of, 95
Bacteriologic examination of organs and cavities, 257
Bacteriolysins, 41
Bacterium acidi lactici, 249
bulgaricum, 250
caucasicus, 250
coli commune, 134
güntheri, 250
lactis acidi, 250

Bacterium prodigiosum, 97
as cancer remedy, 98
saccharobutyricus, 251
syncyanum, 251
termo, 147
tumefaciens, 232
Zopfii, 100
Basic dyes, 48
Beer fermentation, 256
Beggiatoa alba, 228
Bichlorid of mercury, 62
Biedert's method of collecting tubercle bacilli, 116
Bile, lactose-, 76
salt agar, MacConkey's, 75
Biologic activities, 26, 28
Black-leg, 187
Blastomyces, 223
Blastomycetes, 220, 222
Blastomycetic dermatitis, 223
Blender, 44
Blood albumin, solution dried, 78
bacillus typhosus from, 143
bacteria in, 260
coagulum, 74
cultures, 261
specimens, staining, 57, 261
Blood-agar, 76
Blood-serum, 69
coagulation of, 72
human, preparation of, 74
mixture, Löffler's 74
nutrient, preparation of, 72
preservation, in liquid state, 74
sterilization of, 73
test, Gruber-Widal, 140
Bluish-green pus, bacillus of, 171
Boas-Oppler bacillus, 101
Boiled eggs, 78
Boiling as means of purifying water, 238
Bordet's cholera test, 155
Borer, Fränkel's, 244
Boric acid, 264
Botkin's method of cultivating anaërobic bacteria, 84
Bouillon filtrate, Denys', 120
guinea-pig, 78
Bovine farcin du bœuf, 231
tuberculosis, human tuberculosis and, relation, 119
Bowhill's orcein stain, 60
Bread mash, 69
Brill's disease, 219
Broth, nitrate, 70, 71
nutrient, 71
sugar, 71
Brownian movements, 23
Bubonic plague, 190
Buchner's method of cultivating anaërobic bacteria, 84
Budding fungi, 220
Buerger's method of staining capsule, 61
Butter, bacteria in, 254
Buttermilk, bacteria in, 255

Cadaveric alkaloids, 34
Calmette's ophthalmic tuberculin reaction, 121
Cancer remedy, bacterium prodigiosum as, 98
Capsule bacillus of Pfeiffer, 159
of spore, 25
stain, 61
Buerger's method, 61
Hiss' method, 52, 61
of Welch, 53
Carbolfuchsin, 49
Carbolthionin stain, 52
Carriers, cholera, 153
typhoid, 139
Catarrhal conjunctivitis, 258
Cattle-fever, Texas, 208
Cell-contents, 22
Celloidin sacs for animal inoculation, 93
Cell-wall, 22
Cerebral membranes, animal inoculation by, 93
Cerebrospinal meningitis, epidemic, 177
Chancre, soft, 178
Chancroid, 178
Charbon symptomatique, 187, 189
Charcoal sponge, 236
Cheese, bacteria in, 254
Chemic activities, 26
composition, 22
products of bacteria, 29
Chicken cholera, 193

Chlorin, 264
Cholera, 148
  bacteria, 148
  carriers, 153
  chicken, 193
  comma bacillus of, 148
  hog, 133
  red, 152
Chromatin stain, Wright's, for malarial organisms, 204
Chromium trioxid for staining spores, 60
Cladothrices, 227
Cladothrix actinomyces, 228
  dichotoma, 227
Classification, 22
Clostridium butyricum, 251
Coagulating ferments, 30
Coagulation of blood-serum, 72
Coagulum, blood, 74
Cocci, pyogenic, 163
Coley's fluid, 166
Colitis contagiosa, 135
Colon bacillus, 134
    bacillus typhosus and, differentiation, 142
Colon-typhoid group, 133
Colonies, growth and appearances, 87
  impression of, 88
  macroscopic appearance, 87
  microscopic appearance, 88
Combinor, 42
Comma bacillus of cholera, 148
Complement, 40, 42
  deviation of, 42
  fixation of, 42
Completor, 42
Concentrated staining solutions, 48
Condensed milk, bacteria in, 255
Condenser, Abbé's, 44
Conjunctiva, bacteria in, 258
Conjunctivitis, catarrhal, 258
  diplobacillus of, 171
  epidemic, 161
Conradi-Drigalski medium, 76
Copper sulphate, 264
Copula, 40
Corrosive sublimate, 262
Cotton plugs or corks, 66
Cover-glass preparations, 54
Cream, classification of, 248
Crenothrix kühniana, 227
Cresols, 264
Crown gall, 232
Cultivation, 62
  artificial, 62
  of anaërobic bacteria, 82
      Botkin's method, 84
      Buchner's method, 84
      Esmarch's method, 83
      Fränkel's method, 84
      Hesse's method, 83
      Liborius's method, 82
      Park's method, 85
      Roux's method, 84
      Wright's method, 85
Culture-media, inoculation of, 78
  nutrient, preparation of, 67, 70
  reaction, 70
  solid transparent, 69
  sterilization of, 62, 70
Cultures, blood, 261
  fresh egg, 77
  glass slide, 78
  plate, 79
  pure, by boiling, 81
  rolled, 80
  stab, 78
  stroke, 78
  test-tube, 78
  thrust, 78
Cutaneous inoculation of animals, 91
Cutting sections, 56
Cytase, 40
Cytolysins, 40
Cytolytic serum, 40

Decolorizing agents, 49
Denitrification, 245
Denys' B. F. tuberculin, 120
Deodorants, 262
Dermatitis, blastomycetic, 223
Dermo-tuberculin reaction of von Pirquet, 121
Desmon, 40
Development, 21
Deviation of complement, 42
Diastatic ferments, 29

Dieudonné's medium, 77
for spirillum choleræ, 151
Diphtheria, 126
antitoxin of, 131
methods of diagnosis, 130
Neisser's stain for, 52
streptococcus in, 133
toxins of, 131
Diplobacillus of conjunctivitis, 171
Diplococcus intracellularis meningitidis, 177
lanceolatus, 157
pneumoniæ, 155, 157
Discharges, disinfection of, 265
Disinfectants, 62, 262
testing value of, 265
Disinfection, general measures for, 265
of discharges, 265
Distribution, 26
Dourine, 204
Dry heat, 64
Drying specimens, 54
Dum-dum fever, 208
Dunham's modified peptone water, 77
peptone solution for spirillum choleræ, 150
rosalic acid solution, 77
Dyes, acid, 48
anilin, 47, 264
anilin-oil water, 50
basic, 48
Dysentery, 145, 198
bacillary, 199

EAR, bacteria in, 259
Edema, malignant, 184
Eggs, boiled, 78
fresh, cultures, 77
Ehrlich's lateral-chain theory of immunity, 36
Electricity, influence of, on bacteria, 28
Elsner's typhoid medium, 76
Emphysema, phlegmonous, 186
Emphysematous gangrene, 186
Endo medium, 77
Endogenous infection, 31
Endosporous bacteria, 24
Entamœba histolytica, 198
Enteric fever, 135
Epidemic cerebrospinal meningitis, 177
conjunctivitis, 161
Erysipelas, 164
of swine, 194
Esmarch's cubes, 68
method of cultivating anaërobic bacteria, 83
tubes, 80
Estivo-autumnal form of malarial protozoa, 201
Exogenous infection, 31
Extracellular toxins, 31
Eye, inoculation of animals by, 92

FACULTATIVE aërobes, 28
anaërobes, 28
bacteria, 27
Farcin du bœuf, bovine, 231
Farcy-buds, 125
Fat-splitting ferments, 30
Favus, 225
Feces, bacteria in, 260
cholera bacteria in, 153
Fermentation, 29
alcohol, 255
beer, 256
tube, 81
vinegar, 255
Ferments, 29
coagulating, 30
diastatic, 29
fat-splitting, 30
hydrolytic, 30
inverting, 29
proteolytic, 29
Fertility, soil, bacteria and, 244
Filter materials, 236
Pasteur-Chamberland, 237
Petri's sand, 233
Filterable organisms, 218
Filtered water, 236
Filters, 67
Filtration, 265
sterilization by, 67
Finkler-Prior vibrio, 154

Fishing, 88
Fission algæ, 21
  fungi, 21
Fixateur, 40
Fixation of complement, 42
Flagella, 23
  Löffler's mordant for, 51
  stain, with Löffler's mordant, 61
Flagellates, 197
Flask to receive blood-serum, 72
Flügge's potato bacillus, 98
Fluorescence, 30
Food, bacteria in, 246
Foods as source of infection, 255
Foot, Madura, 230
Formaldehyd, 263
  solid, 264
Fowl septicemia, 193
Fractional sterilization of Tyndall, 65
Fränkel's borer, 244
  method of cultivating anaërobic bacteria, 84
  of staining tubercle bacilli, 115
  pneumococcus, 157
Fresh egg cultures, 77
Fuchsin-lactose-agar, 77
Fungi, budding, 220
  fission, 21
  ray-, 228
  staining, Unna's method, 61
  thrush, 222

Gabbet's acid blue, 51
  modification of Fränkel's method of staining tubercle bacilli, 115
Gametes, 200
Gangrene, emphysematous, 186
Gangrenous mastitis of sheep, 196
Gas phlegmons, 186
Gas-formation, 30
Gelatin, 69
  nutrient, 71
  potato, 76
Gelatinous membrane, 22
Genito-urinary passages, bacteria in, 260
Germicides, 261, 262
Germination, 25
Giemsa's stain, 53
Glanders, 124
Glass plating, 80
  slide cultures, 78
Glossina morsitans, 208
  palpalis, 208
Glycerin-agar, 69
Gonococcus, 174
  allied varieties, 176
  Neisser, 174
  Neisser's stain for, 175
  Wertheim's medium for, 78
Gram's iodin solution, 51
  method of double staining, 58
  of tissue staining, 58
Granulobacillus immobilis, 187
Growth, 27
Gruber-Widal blood-serum test, 140
Guinea-pig bouillon, 78
Guinea-pigs, 91
Günther's stain for blood specimens, 261

Hands, sterilization of, 264
Hanging block, 47
  drop, 46
Haptophore group, 37
Hay bacillus, 100
Heat, 49
  as disinfectant, 63
  as germicide, 262
  dry, 64
  influence of, on bacteria, 28
  moist, 64
Hemolysins, 41
Hemolysis, 41
Hemorrhagic septicemia, 190
  bacteria of, 192
Herpes tonsurans, 225
Herpetomonas, 208
Hesse's medium for typhoid, 75
  method of collecting bacteria from air, 233
  of cultivating anaërobic bacteria, 83
Hiss' capsule stain, 52, 61
  medium for plating, 75
Hoffman, pseudobacillus of, 129
Hog cholera, 133
Homogeneous system, 43

Host, susceptibility of, 33
Hot-air sterilizer, 63
Hueppe's fresh egg cultures, 77
Hydrogen dioxid, 264
Hydrolytic ferments, 30
Hydrophobia, 209
Hygienic laboratory phenol coefficient, 265
Hypersensitiveness, 42
Hypersusceptibility, 42
Hyphomycetes, 223

Ice cream, 248
Immunity, 34, 35
  acquired, 35
    active, 35
    passive, 36
  Ehrlich's lateral-chain theory, 36
  from intentional infection or intoxication, 35
  lock and key theory, 39
  Metchnikoff's phagocytic theory of, 36
  natural, 35
  theories of, 36
  unit, 133
Impression of colonies, 88
Incubator, 74
Index, opsonic, 41
    negative phase, 41
    positive phase, 41
India ink method of identifying spirochæta pallida, 211
Indol reaction in cholera, 152
Infantile paralysis, epidemic, 218, 219
Infection, 30, 31
  avenues of, 32
  cardinal conditions for, 32
  depending on quantity of bacteria, 32
  endogenous, 31
  exogenous, 31
  foods as source of, 255
  mixed, 33
  pathogenesis, 31
  sources of, 31
  susceptibility to, 33
  virulence of, 32
Infective bacteria, 33
Influenza, 160
Infusoria, 197, 198
Inoculating potatoes, manner of, 68
Inoculation, animal, 91. See also *Animal inoculation.*
  of culture-media, 78
Insecticides, 263
Insoluble toxins, 31
Inspissator for blood-serum, 73
Instruments, sterilization of, 45
Intensifiers, 48
Intestine, bacteria in, 259
Intracellular toxins, 31
Intraduodenal animal inoculation, 93
Intraperitoneal injections of animals, 92
Intratracheal inoculation of animals, 93
Intravenous injections of animals, 92
Inverting ferments, 29
Iodin, 264, 265
  as used in Gram's method, 49
  solution, Gram's, 51
Iris blender, 44

Jackson bile media, 242
Jenner's stain, 53
    for malarial organisms, 203

Kala-azar, 208
King's solution dried blood albumin, 78
Klatsch preparations, 88
Klebs-Löffler bacillus, 126
Klein's method of staining spores, 60
Koch's alkaline methylene-blue, 50
  bacillen emulsion, 120
  rules in regard to bacterial cause of disease, 94
Koch-Weeks bacillus, 161
Kühne's method of staining spores, 60
  stain, 51

LABORATORY, small requirements of, 85
Lactose litmus agar, 72
Lactose-bile, 76
Lateral-chain theory of immunity, Ehrlich's 36
Law, Weigert's, 38
Leishman-Donovan bodies, 208
Leishman's stain, 53
Lens, oil-immersion, 43
Lepra bacillus, 122
Leprosy, 122
Leptothrix buccalis, 228
gigantea, 228
innominata, 228
maxima, 228
Liborius's method of cultivating anaërobic bacteria, 82
Life cycle of malarial sporozoa, 199
of protozoa, 198
Light, influence of, on bacteria, 28
Lime, 265
Litmus agar, lactose, 72
Lock and key theory of immunity, 39
Locomotion, 23
Löffler's alkaline methylene-blue, 50
blood-serum mixture, 74
mordant for flagella, 51, 61
stain for tissues, 59
Luetin reaction, 216
Lye, soda, 263
Lysis, 42

MACCONKEY'S bile salt agar, 75
Macrocytase, 36
Macrogamete, 201
Macrophages, 36
Madura foot, 230
Mal de pis, 196
Malarial organisms, methods of examination for, 203
protozoa, estivo-autumnal form, 201
forms of, 201
quartan form, 201
tertian form, 201
sporozoa, life cycle of, 199
Malignant edema, 184
pustule, 105
tertian form of malarial protozoa, 201
Mallein, 126
Malta fever, 162
Mash, bread, 69
potato, 68
Mastigophora, 197
Mastitis, gangrenous, of sheep, 196
Measles, 219
Media, culture-, inoculation of, 78
nutrient, preparation of, 67
preparation of, 70
reaction of, 70
solid transparent, 69
sterilization of, 70
Mediterranean fever, 162
Membrane, gelatinous, 22
Meningitis, epidemic cerebro-spinal, 177
Meningococcus, 174, 177
Mercuric chlorid, 262
Mercury, bichlorid of, 62
Merozoites, 199
Metals, salts of, 263, 264
Metchnikoff's theory of immunity, 36
Methylene-blue, alkaline, 50
Microbe en huit, 193
Micrococcus albicans, 176
cereus albus, 169
flavus, 169
cholera gallinarum, 193
citreus, 176
gonorrhœæ, 174
melitensis, 162
meningitidis, 177
of mal de pis, 196
of sputum septicemia, 157
pyogenes citreus, 169
tenuis, 169
subflavus, 176
tetragenus, 170
ureæ, 102
Microcytase, 36
Microgametes, 201
Microphages, 36
Microscope, 43
Microsporon furfur, 224, 226

Milk as source of contagion, 253
bacillus coli in, Wisconsin test for, 255
bacteria in, 246, 249
classification of, 247
condensed, bacteria in, 255
culture-medium, 77
examination of, 252
for tubercle bacilli, 116
pasteurized, 247, 253
pure, 246
raw, 247
red, 252
yellow, 252
Milzbrand bacillus, 105
Mixed infection, 33
Moist heat, 64
Moisture, influence of, on bacteria, 28
Molds, 220, 221
examination of, 226
true, 223
Morax-Axenfeld diplobacillus of conjunctivitis, 171
Mordant, Löffler's, for flagella, 51
for flagella, 61
Mordants, 48
Moro's tuberculin test, 121
Mouse septicemia, 195
Mouth, bacteria in, 258
white, 222
Mucor mucedo, 224
Mycetoma, 230
Mycobacterium tuberculosis, 110

NAGANA, 204, 206
Naphthalin, 264
Nasal cavity, bacteria in, 259
Needles, platinum, 45
sterilization of, 79
Negri bodies, 209
Neisser's gonococcus, 174
stain for diphtheria, 52
for gonococcus, 175
Nicolle's stain, 52
Nitragin, 246
Nitrate broth, 70, 71
Nitrification, 245
by bacteria, 29
Nitro-bacterine, 246
Nocardia farcinica, 231
Noguchi's luetin reaction, 216
Novy's jars, 85
Nutrient agar, 71
blood-serum, preparation of, 72
broth, 71
culture-media, preparation, 67, 70
gelatin, 71

OBLIGATE aërobes, 28
anaërobes, 28
Odors from bacteria, 30
Oïdiomycosis, 223
Oïdium, 221, 223
albicans, 222
coccidioides, 223
lactis, 222
Oil-immersion lens, 43
Oöcysts, 201
Ophthalmic tuberculin reaction of Calmette, 121
Opsonic index, 41
negative phase, 41
positive phase, 41
Opsonins, 41
Orcein stain, Bowhill's, 60
Origin, 26
Oxidation by bacteria, 29
Oxygen, influence of, on life and growth of bacteria, 27

PAPPENHEIM'S method for staining tubercle bacilli in urine, 115
Paracolon bacillus, 143
Paralysis, epidemic infantile, 218, 219
Parasites, 27
Parasitic bacteria, 27
thrush, 222
Paratyphoid bacilli, 143
Park's method of cultivating anaërobic bacteria, 85
Pasteur-Chamberland filter, 237
Pasteurized milk, 247, 253
Pathogenic bacteria, 29, 97
table of, 287–303
yeasts, 222
Pear blight, 232

Penicillium glaucum, 223
Peptone water, modified Dunham, 77
Pertussis, 161
Pest, 190
Petri dish, 80
  method of collecting bacteria from air, 232, 233
  sand-filter, 233
Pfeiffer's capsule bacillus, 159
  cholera reaction and agglutination, 153, 154
Phagocytes, 36
Phagocytic theory of immunity, Metchnikoff's, 36
Phenol, 263
  coefficient, Hygienic Laboratory, 265
  solutions, 50
Phlegmonous emphysema, 186
Phlegmons, gas, 186
Phosphorescence, 30
Pigmentation, 30
Pink eye, 161, 258
Piroplasma bigeminum, 208
  bovis, 208
  hominis, 209
Pityriasis versicolor, 226
Plague, bubonic, 190
Plant diseases due to bacteria, 231
Plants, splitting, 21
Plasmodium falciparum, 201
  malariæ, 201
  vivax, 201
Plate cultures, 79
  method of examining milk for bacteria, 253
Plating, glass, 80
  streaked surface, 79
Platinum needles, 45
Pneumococcus, 157
  Fränkel's, 157
Pneumonia, 155
  bacteria in, 155
Poliomyelitis, infantile, 218, 219
Potassium iodid medium, 76
  permanganate, 264
Potato as culture-media, 67
  as medium, 67
  bacillus of Flügge, 98
  mash, 68
Potatoes, manner of inoculating, 68
  test-tube, 68
Potato-gelatin, 76
Precipitin serum, 40
Precipitins, 39
Prescott-Breed method of examining milk for bacteria, 252
Preservatives, 262
Protein contents of bacterial cell, 29
Proteolytic ferments, 29
Proteus mirabilis, 147
  Zenkeri, 148
Protozoa, 197
  life-cycle of, 198
  malarial, estivo-autumnal form, 201
    forms of, 201
    quartan form, 201
    tertian form, 201
Pseudobacillus of Hoffman, 129
Pseudodiphtheria, 129
Ptomains, 29, 34
Puerperal fever, 165
Pure cultures by boiling, 81
Pus, bluish-green, bacillus of, 171
Pustule, malignant, 105
Putrefaction, 30
Pyemia, 33
Pyocyanin, 173
Pyogenic bacteria, 33
  cocci, 163

QUARTAN form of malarial protozoa, 201
Quarter-evil, 187

RABIES, 209
Rauschbrand, 187, 189
Ray-fungus, 228
Reaction. See *Test.*
  of culture-media, 70
Reactivity, altered, 42
Receptors, 36
  free, 41
  of first order, 36
  of second order, 36
  of third order, 37

Red milk, 252
Reduction by bacteria, 29
Relapsing fever, 217
Removing excess of stain, 55
Rennet curd cheese, 255
Reproduction, 24
Rhinoscleroma, 159
Rideal-Walker standard for disinfectants, 265
Ring-worm, 225
Rolled cultures, 80
Romanowsky's stain, 53
Röntgen rays, influence of, on bacteria, 28
Root bacillus, 99
Rosalic acid solution, Dunham's, 77
Rotz-bacillus, 124
Rouget du porc, 194
Roux's double stain, 52
  method of cultivating anaërobic bacteria, 84
  test-tube, 68

Saccharomyces albicans, 221
  cerevisiæ, 220
  mycoderma, 221
  niger, 221
  rosaceus, 221
Saccharomycetes, 220
Salicylic acid, 264
Salts of metals, 263, 264
Sand-filter, Petri's, 233
Saprophytes, 27
Sarcina, 103
  aurantica, 104
  lutea, 103
  ventriculi, 104
Sarcodina, 197
Schizogony, 199
Schizomycetes, 21
Schizophyceæ, 21
Schizophyta, 21
Schweinerotlaufbacillus, 194
Sedgwick's expanded tube for air-examination, 233
Sedgwick-Tucker method of collecting bacteria from air, 233
Sedimentation test in typhoid fever, 142
Septicemia, 33
  fowl, 193
  hemorrhagic, 190
    bacteria of, 192
  mouse, 195
  sputum, micrococcus of, 147
Serum, antistreptococcic, 166
  antitoxic, 39
  antituberculous, 122
  blood-, 69
    coagulation of, 72
    human, preparation of, 74
    nutrient, preparation of, 72
    preservation of, in liquid state, 74
    sterilization of, 73
  cytolytic, 40
  precipitin, 40
  test, Wassermann, 42
Sewage bacteria, examination for, 240
  bacterial treatment of, 243
Sewer air, bacteria in, 235
Sexual cycle in mosquito, 201
Sheep, gangrenous mastitis of, 196
Shiga bacillus, 199
Side-chain theory of immunity, Ehrlich's, 36
Skin, bacteria on, 257
Sleeping sickness, 205, 207
Small-pox, 218
Smear culture, 78
Smegma bacillus, 123
Smith's fermentation tube, 81
Soap, 264
Soda lye, 263
Soil bacteria in, 232, 243
  examination of, 232, 243
  fertility, bacteria and, 244
Soluble toxins, 31
Soor, 222
Spatula for lifting sections, 57
Specimens, drying, 54
Spirillum, 103
  aquatilis, 154
  berolinense, 154
  bonhoffii, 154
  choleræ, 148
    allied varieties, 154
    products, 152
  danubicum, 154

Spirillum dunbarii, 154
milleri, 154
obermeieri, 217
of relapsing fever, 217
of Wernicke, 154
rubrum, 103
schuylkilliensis, 154
weibeli, 154
Spirochæta pallida, 209
Spironema pallidum, 209
Splenic fever, 105
Splenomegaly, tropical, 208
Splitting plants, 21
Sporangium, 224
Spore contents, 25
formations, 24
requisites for, 25
Spores, resistance of, 26
staining, 59
Klein's method, 60
Kühne's method, 60
Weigert's method, 61
Sporidium vaccinale, 219
Sporocyte, 199
Sporogenic bodies, 25
Sporogony, 199
Sporozoa, 197, 198
malarial, life cycle of, 199
Sporozoïtes, 201
Sputum septicemia, micrococcus of, 157
Stab culture, 78
Stain, alkaline, 49
Bowhill's orcein, 60
capsule, 61
Buerger's method, 61
Hiss' method, 61
carbolthionin, 52
flagella, with Löffler's mordant, 61
Fränkel's, for tubercle bacilli, 115
Giemsa's, 53
Günther's, for blood specimens, 261
Hiss' capsule, 52, 61
Jenner's, 53
for malarial organisms, 203
Koch's, 50
Kühne's, 51
Leishman's, 53
Stain, Löffler's, 50
for tissues, 59
Neisser's, for diphtheria, 52
for gonococcus, 175
Nicolle's, 52
removing excess of, 55
Romanowsky's, 53
Roux's double, 52
Welch's capsule, 53
Wright's, 53
for malarial organisms, 204
Ziehl-Neelsen, 50
Staining, 47
blood specimens, 57, 261
fungi, Unna's method, 61
general method, 54
Gram's double method, 58
for tissues, 58
malarial organisms, 203
of tissue sections, 54, 56, 57
solutions, 48
compound, 48
concentrated, 48
formulas of, 49
stock, 48
special methods, 58
spores, 59
Klein's method, 60
Kühn's method, 60
Weigert's method, 61
tubercle bacilli in sputum, 117
Staphylococcus, 23
epidermidis albus, 168
pyogenes, 164
albus, 168
aureus, 166
Steam, superheated, 263
Stegomyia fasciata, 219
Sterilization by filtration, 67
fractional, of Tyndall, 65
of blood-serum, 73
of culture-media, 62, 70
of hands, 264
of instruments, 45
of needles, 79
Sterilizer, Arnold's steam, 64
hot-air, 63
Stewart-Slack method of examining milk for bacteria, 252
Stock staining solutions, 48
Stomach, bacteria in, 259

Streaked surface plating, 79
Streptococcus, 23
  acidi lactici, 250
  erysipelatis, 164
  in diphtheria, 133
  lanceolatus, 157
  puerperalis, 165
  pyogenes, 164
Streptothrices, 227
Streptothrix actinomyces, 228
  farcinica, 231
  maduræ, 230
Stroke culture, 78
Structure, 21, 22
Subcutaneous inoculation of animals, 92
Substance sensibilatrice, 40
Sugar broths, 71
Sulphur dioxid, 264
Sulphurous acid gas, 264
Suppurative bacteria, 33
Surra, 204
Susceptibility, 33
  acquired, 33
  inherited, 33
  natural, 33
Swine, erysipelas of, 194
Symptomatic anthrax, 187
Syphilis, 209
  luetin reaction in, 216
  Wassermann reaction in, 211
    results, 216
    Noguchi modification, 214

Temperature, influence of, on life and growth of bacteria, 27
Tertian form of malarial protozoa, 201
Test, agglutination, for tubercle bacilli, 121
  in cholera, 152
  in typhoid fever, 140
  Pfeiffer's cholera, 153, 154
  Bordet's cholera, 155
  Gruber-Widal blood-serum, 140
  luetin, 216
  Pfeiffer's cholera, 153, 154
  sedimentation, in typhoid fever, 142
Test, tuberculin, Calmette's ophthalmic, 121
  Moro's, 121
  von Pirquet's, 121
  Wassermann, in syphilis, 211
    Noguchi's modification, 214
    results, 216
  Widal, in typhoid fever, 140
  Wisconsin, for bacillus coli in milk, 255
Test-tube, 66
  cultures, 78
  potatoes, 68
  Roux's, 68
Tetanolysin, 183
Tetanospasmin, 182
Tetanus, 180
  antitoxin, 183
    unit for, 183
Texas cattle-fever, 208
Thermostat for blood-serum, 73
Thrush fungus, 222
  parasitic, 222
Thrust-culture, 78
Tick fever, African, 218
Tinea, 225, 226
Tissue preparations, 56
Torula cerevisiæ, 220
Toxalbumins, 30, 34
Toxemia, 33
Toxins, 30, 31, 34
  of diphtheria, 131
  extracellular, 31
  insoluble, 31
  intracellular, 31
  nature of, 34
  soluble, 31
Toxoid of diphtheria, 131
Toxone of diphtheria, 131
Toxophore group, 37
Treponema pallidum, 209
Trichophyton tonsurans, 224, 225
Tricresol, 263
Tropical splenomegaly, 208
Trypanosoma, 204
  brucei, 206
  castellani, 207
  equiperdum, 208
  Evansi, 208
  hominis, 207
  lewisi, 205

Unna-Kerr's organisms, 218
Unna's borax methylene blue, 51
  method for staining fungi, 61
Urethra, bacteria in, 200
Urine, bacillus coli in, 102
  bacteria in, 102, 260
  examination of, for tubercle bacilli, 115

Vaccines, 95
  antistreptococcic, 166
  Pasteur's, 251
  tuberculin, 222
Vaginal secretion, 200
  bacteria in, 200
Vaginitis, 200
Vegetable parasites, 1
Vegetative cells, 234
  forms, 235
    sedimentation test in, 242
    Vital Stain in, 242
  medium, Eisner's, 71
    for typhoid, 73
Verruga, 232
Vibrio Metschnikovi, 221
  Proteus, 221
Vienna, Pasteur Institute at, 251
Virulence, 23
Vital Stain in vegetative forms of bacteria, 242
Volatile substances, 23
Volutin granules, 23
Von Fleisch's method of staining tubercle bacilli, 111
Von Pirquet's cutaneous tuberculin test, 222

Wassermann reaction in syphilis, 211
  Noguchi modification, 214
  results, 221
Water, acidity of, 236
  bacillus coli in, examination for, 242
    typhosus in, diagnostic points of, 242
  bacteria in, method of examination, 238
    varieties, 238
  boiling of, as means of purifying, 238
  cholera bacillus in, 152
  examination of, 232, 235, 238
  filtered, 236
  purity of, 235
  well, 236
Weak solutions, 30
Weigert's law, 38
  method of staining spores, 61
Welch's capsule stain, 53
Well water, 236
Wernicke's spirillum, 154
Wertheim's medium for gonococcus, 78
White mouth, 222
Whooping-cough, 161
Widal reaction in typhoid fever, 140
Wire cage, 66

Wisconsin test for bacillus coli in milk, 255
Wolfhügel's counter, 243
Woolsorter's disease, 109
Wright's chromatin stain for malarial organisms, 204
method of cultivating anaërobic bacteria, 85
stain, 53
Wurzel bacillus, 99

YAWS, 217
Yeasts, 220
examination of, 226
pathogenic, 222
Yellow fever, 219
milk, 25

ZIEHL-NEELSEN stain, 50
Zoöglea, 23

SAUNDERS' BOOKS

on

Nervous and Mental Diseases, Children, Hygiene, Nursing, and Medical Jurisprudence

W. B. SAUNDERS COMPANY

West Washington Square — **Philadelphia**

9, Henrietta Street — **Covent Garden, London**

Our Handsome Complete Catalogue will be Sent on Request

Dercum's Mental Diseases

Mental Diseases. A Clinical Manual. By FRANCIS X. DERCUM, M. D., Ph. D., Professor of Nervous and Mental Diseases at Jefferson Medical College, Philadelphia. Octavo of 425 pages. Cloth, $3.00 net.

JUST ISSUED—FOR THE FAMILY PHYSICIAN

Dr. Dercum's work is along entirely new lines. It is *strictly clinical.* It is a book on mental diseases *really useful* to the *family physician*—a book that tells you how to diagnose, how to treat—either at home or in an institution—all classes of mental diseases ; tells you in a clear, simple way. There is no other book just like it. You are introduced at once into a *clinical* consideration of the various primary forms of mental disease, first taking up the affections you meet in your daily practice—delirium, confusion, stupor. Melancholia, mania, the insanities of early life, paranoia, the neurasthenic-neuropathic disorders, and the dementias follow in their turn. Then the mental disturbances of the infections, intoxications, metabolic disorders, visceral diseases, and diseases of the nervous system are taken up. An important section is that on the *insanities of pregnancy, parturition, and the puerperium.*

Church and Peterson's Nervous *and* Mental Diseases

Nervous and Mental Diseases. By ARCHIBALD CHURCH, M.D., Professor of Nervous and Mental Diseases and Head of Neurologic Department, Northwestern University Medical School, Chicago; and FREDERICK PETERSON, M. D., Professor of Psychiatry in Columbia University, New York. Octavo, 934 pages, with 341 illustrations. Cloth, $5.00 net; Half Morocco, $6.50 net.

THE NEW (7th) EDITION

For this new seventh edition the entire work has been most thoroughly revised. To show with what thoroughness the authors have revised their work, we point out that in the nervous section alone over one hundred and fifty interpolations have been made, and, in addition, well over three hundred minor corrections. The section on Mental Diseases has been wholly rearranged to conform to the latest classification, some obsolete matter struck out, and much new matter added.

American Journal of the Medical Sciences

"This edition has been revised, new illustrations added, and some new matter, and really is two books. . . . The descriptions of disease are clear, directions as to treatment definite, and disputed matters and theories are omitted. Altogether it is a most useful text-book."

Brill's Psychanalysis

Psychanalysis: ITS THEORIES AND PRACTICAL APPLICATION. By A. A. BRILL, M. D., Clinical Assistant of Psychiatry and Neurology at Columbia University Medical School. 12mo of 337 pages. Cloth, $3.00 net.

This one volume gives you the practical application of all Freud's theories, and from the pen of a man thoroughly competent. Psychanalysis is the only system of psychotherapy that deals with the neuroses as entities instead of treating symptoms as do hypnotism, suggestion, and persuasion. The results of psychanalysis are most effective.

Just Out—The New "Dorland"

New (7th) Edition—5000 New Words

The American Illustrated Medical Dictionary. A new and complete dictionary of the terms used in Medicine, Surgery, Dentistry, Pharmacy, Chemistry, Veterinary Science, Nursing, and kindred branches; with over 100 new and elaborate tables and many handsome illustrations. By W. A. NEWMAN DORLAND, M. D. Large octavo, 1107 pages, bound in full flexible leather, $4.50 net; with thumb index, $5.00 net.

Dorland's Dictionary defines hundreds of the newest terms not defined in any other dictionary—bar none. It gives the capitalization and pronunciation of all words. It makes a feature of the derivation or etymology of the words. In "Dorland" every word has a separate paragraph, thus making it easy to find a word quickly. The tables of arteries, muscles, nerves, veins, etc., with accompanying pictures in colors, are of the greatest help in assembling anatomic facts. In "Dorland" every word is given its definition—a definition that *defines* in the fewest possible words.

Howard A. Kelly, M. D., *Johns Hopkins University, Baltimore.*

"Dr. Dorland's dictionary is admirable. It is so well gotten up and of such convenient size. No errors have been found in my use of it."

Goodnow's First-Year Nursing

Illustrated

First-Year Nursing. By MINNIE GOODNOW, R. N., formerly Superintendent of the Women's Hospital, Denver. 12mo of 325 pages, illustrated. Cloth, $1.50 net.

Miss Goodnow's work deals *entirely* with the *practical* side of first-year nursing work. It is the *application* of text-book knowledge. It tells the nurse *how* to do those things she is called upon to do in her first year in the training-school—the *actual ward work.*

Roberts' Bacteriology and Pathology for Nurses

Bacteriology and Pathology for Nurses. By JAY G. ROBERTS, PH. G., M. D., Oskaloosa, Iowa. 12mo of 206 pages, illustrated. Cloth, $1.25 net.

This new work is practical in the strictest sense. Written specially for nurses, it confines itself to information that the nurse should know. All unessential matter is excluded. The style is concise and to the point, yet clear and plain. The text is illustrated throughout.

Grulee's Infant Feeding

Infant Feeding. By CLIFFORD G. GRULEE, M. D., Assistant Professor of Pediatrics at Rush Medical College. Octavo of 295 pages, illustrated, including 8 in colors. Cloth, $3.00 net.

COLORED PLATES OF STOOLS

After reviewing the scientific principles bearing on the subject, Dr. Grulee goes into the *actual application* of them. He tells you *how* to feed the infant. He tells you—and *shows* by clear illustrations—the *technic* of giving the child the breast. Then artificial feeding is thoughtfully presented, including a number of simple formulas. The colored illustrations showing the actual shapes and appearances of stools are extremely valuable.

Pediatrics

"This work is the *last word* on infant feeding. It is a work that should be in the hands of every physician who is called upon to give advice where the subject of feeding babies and children is concerned."

Bergey's Hygiene

The Principles of Hygiene: A Practical Manual for Students, Physicians, and Health Officers. By D. H. BERGEY, A. M., M. D., Assistant Professor of Bacteriology, University of Pennsylvania. Octavo of 529 pages, illus. Cloth, $3.00 net.

FOURTH EDITION

This book is intended to meet the needs of students of medicine in the acquirement of a knowledge of those principles upon which modern hygienic practices are based, and to aid physicians and health officers in familiarizing themselves with the advances made in hygiene and sanitation in recent years.

Buffalo Medical Journal

"It will be found of value to the practitioner of medicine and the practical sanitarian; and students of architecture, who need to consider problems of heating, lighting, ventilation, water supply, and sewage disposal, may consult it with profit."

Kerr's Diagnostics of Diseases of Children

Diagnostics of the Diseases of Children. By LEGRAND KERR, M. D., Professor of Diseases of Children, Brooklyn Post-graduate Medical School, Brooklyn. Octavo of 542 pages, fully illustrated. Cloth, $5.00 net; Half Morocco, $6.50 net.

FOR THE PRACTITIONER

In Dr. Kerr's work the *objective symptoms* are particularly emphasized. Differential diagnosis is discussed from the very earliest symptoms. The physician will find the many original illustrations a source of much information and help.

New York State Journal of Medicine

"The illustrations are excellent and numerous. It will meet the needs of the great mass of physicians who treat the diseases of infancy and childhood."

Kerley's Treatment of Diseases of Children

Treatment of the Diseases of Children. By CHARLES GILMORE KERLEY, M. D., Professor of Diseases of Children, New York Polyclinic School and Hospital. Octavo of 629 pages, illustrated. Cloth, $5.00 net; Half Morocco, $6.50 net.

SECOND EDITION

This work has been prepared for the physician engaged in general practice. The author *presents all the modern methods of management and treatment in greater detail than any other work on the subject* heretofore published. Every method has been thoroughly tried and its value as a therapeutic measure proved. There is an excellent illustrated chapter on *Gymnastic Therapeutics.*

The British Medical Journal

"Dr. Kerley's book is one of the best on the subject that has come under our notice. All through it shows evidence of ripe experience and sound judgment."

Ruhrah's Diseases of Children

A Manual of Diseases of Children. By JOHN RUHRAH, M. D., Professor of Diseases of Children, College of Physicians and Surgeons, Baltimore. 12mo of 534 pages, fully illustrated. Flexible leather, $2.50 net.

THE NEW (3d) EDITION

The third edition makes this work more than ever the ideal desk book for the general practitioner. Although there have been added over one hundred pages of new matter and some sixty new illustrations, the book remains of a handy size and is still flexible. Among some of the amplified articles are those on the examination of sick children, food intoxications, bronchopneumonia, examination of the heart, and the nervous system. The section on therapeutics has been very largely rewritten, and that on the infectious diseases entirely rewritten. There has been added a table of doses, instructions for summer, care of the mentally deficient, the blind, and the deaf.

American Journal of the Medical Sciences

"Treatment has been satisfactorily covered, being quite in accord with the best teaching, yet withal broadly general and free from stock prescriptions."

Griffith's Care of the Baby

The Care of the Baby. By J. P. CROZER GRIFFITH, M. D., Clinical Professor of Diseases of Children, University of Pennsylvania. 12mo of 455 pages, illustrated. Cloth, $1.50 net.

THE NEW (5th) EDITION

New York Medical Journal

"We are confident if this little work could find its way into the hands of every trained nurse and of every mother, infant mortality would be lessened by at least fifty per cent."

Sanders' Nursing

Modern Methods in Nursing. By GEORGIANA J. SANDERS, formerly Superintendent of Nurses at the Massachusetts General Hospital. 12mo of 881 pages, with 227 illustrations. Cloth, $2.50 net.

COMPLETE

Miss Sanders' book gives only *modern* methods. Then it gives the *details* of nursing *operation cases*, both in the hospital and in the home. The thorough way in which *ward work* is taken up makes her book indispensable for teaching purposes. In giving directions for mustard baths, poultices, etc., the *quantities* are given exactly. This is an important point often overlooked.

Stoney's Nursing

Practical Points in Nursing: for Nurses in Private Practice. By EMILY M. A. STONEY, formerly Superintendent of the Training School for Nurses at the Carney Hospital, South Boston, Mass. 495 pages, fully illustrated. Cloth, $1.75 net.

THE NEW (4th) EDITION

In this volume the author explains the entire range of *private* nursing as distinguished from *hospital* nursing, and the nurse is instructed how best to meet the various emergencies of medical and surgical cases when distant from medical or surgical aid or when thrown on her own resources. An especially valuable feature will be found in the direction how to *improvise* everything ordinarily needed in the sick-room.

Stoney's Technic for Nurses

Bacteriology and Surgical Technic for Nurses. By EMILY M. A. STONEY, Carney Hospital, South Boston. Revised by FREDERIC R. GRIFFITH, M. D., Surgeon, N. Y. 12mo, 311 pages, illustrated. $1.50 net. **New (3d) Edition**

Hoxie and Laptad's Medicine for Nurses

Just Issued
New (2d) Edition

MEDICINE FOR NURSES AND HOUSEMOTHERS. By GEORGE HOWARD HOXIE, M. D., Physician to the German Hospital, Kansas City; and PEARL L. LAPTAD, formerly Principal of Training-School, University of Kansas. 12mo of 351 pages, illustrated. Cloth, $1.50 net.

This work is truly a practice of medicine for the nurse, enabling her to recognize any signs and changes that may occur between visits of the physician, and, if necessary, to combat them until the physician's arrival. This information the author presents in a way most acceptable, particularly emphasizing the nurse's part. There are also special chapters on the diseases of the eye, ear, nose, and throat, venereal diseases, nervous and mental diseases, surgical nursing, emergency measures.

"This book has our unqualified approval."—*Trained Nurse and Hospital Review.*

McCombs' Diseases of Children for Nurses

Second Edition

DISEASES OF CHILDREN FOR NURSES. By ROBERT S. MCCOMBS, M. D., Instructor of Nurses at the Children's Hospital of Philadelphia. 12mo of 460 pages, illustrated. Cloth, $2.00 net.

Dr. McCombs has given a short but clear description of each disease found in infancy and childhood, so that the nurse will be enabled to know what symptoms to expect and what complications to guard against.

"We have needed a good work on children's diseases adapted for nurses' use, and this volume admirably fills the want."—*National Hospital Record.*

Wilson's Obstetric Nursing

Second Edition

A REFERENCE HAND-BOOK OF OBSTETRIC NURSING. By W. REYNOLDS WILSON, M. D., Visiting Physician to the Philadelphia Lying-in Charity. 32mo of 258 pages, illustrated. Flexible leather, $1.25 net.

"Every page emphasizes the nurse's relation to the case."—*American Journal of Obstetrics.*

Fruhwald and Westcott on Children

DISEASES OF CHILDREN. By PROF. DR. F. FRÜHWALD, of Vienna. Edited by THOMPSON S. WESTCOTT, M.D., University of Pennsylvania. Octavo, 533 pages, 176 illustrations. Cloth, $4.50 net.

Boyd's State Registration for Nurses

STATE REGISTRATION FOR NURSES. By LOUIE CROFT BOYD, R.N., Graduate Colorado Training-school for Nurses. Octavo of 42 pages. 50 cents net.

Aikens' Primary Studies for Nurses

New (2d) Edition

PRIMARY STUDIES FOR NURSES: A Text-Book for First-year Pupil Nurses. By CHARLOTTE A. AIKENS, formerly Director of Sibley Memorial Hospital, Washington, D. C. 12mo of 437 pages, illustrated. Cloth, $1.75 net.

This work brings together in concise form well-rounded courses of lessons in all subjects which, with practical nursing technic, constitute the primary studies in a nursing course.

Trained Nurse and Hospital Review

"It is safe to say that any pupil who has mastered even the major portion of this work would be one of the best prepared first-year pupils that ever stood for examination."

Aikens' Clinical Studies for Nurses

New (2d) Edition

CLINICAL STUDIES FOR NURSES. By CHARLOTTE A. AIKENS, formerly Director of Sibley Memorial Hospital, Washington, D. C. 12mo of 569 pages, illustrated. Cloth, $2.00 net.

This new work is written along the same lines as Miss Aikens' earlier work on "Primary Studies," to which it is a companion volume. It takes up all subjects taught during the second and third years and takes them up in a concise, forceful way.

Dietetic and Hygienic Gazette

"There is a large amount of practical information in this book which the experienced nurse, as well as the undergraduate, will consult with profit. The illustrations are numerous and well selected."

Aikens' Training-School Methods and Head Nurse

HOSPITAL TRAINING-SCHOOL METHODS AND THE HEAD NURSE. By CHARLOTTE A. AIKENS, formerly Director of Sibley Memorial Hospital, Washington, D. C. 12mo of 267 pages. Cloth, $1.50 net.

Trained Nurse and Hospital Review

"There is not a chapter in the book that does not contain valuable suggestions."

Aikens' Hospital Management

HOSPITAL MANAGEMENT. By CHARLOTTE A. AIKENS, formerly Director of Sibley Memorial Hospital, Washington, D. C. 12mo of 488 pages, illustrated. Cloth, $3.00 net.

The Medical Record

"Tells in concise form exactly what a hospital should do and how it should be run, from the scrubwoman up to its financing. A valuable addition to our literature on this subject."

Register's Fever Nursing

A TEXT-BOOK ON PRACTICAL FEVER NURSING. By EDWARD C. REGISTER, M. D., Professor of the Practice of Medicine in the North Carolina Medical College. 12mo of 352 pages. Cloth, $2.50 net.

"Nurses will find this book of great value in this practical branch of their work."—*Trained Nurse and Hospital Review.*

Hecker, Trumpp, and Apt on Children

ATLAS AND EPITOME OF DISEASES OF CHILDREN. By Dr. R. HECKER and Dr. J. TRUMPP, of Munich. Edited, with additions, by ISAAC A. APT, M. D., Professor of Pediatrics, Northwestern Medical School, Chicago. With 48 colored plates, 144 text-cuts, and 453 pages of text. Cloth, $5.00 net.

Aikens' Home Nurse's Hand-Book

HOME NURSE'S HAND-BOOK. BY CHARLOTTE A. AIKENS. 12mo of 276 pages, illustrated. Cloth, $1.50 net.

The point about this work is this: It tells you and *shows* you just how to do those little but important things often omitted from other nursing books. "Home Treatments" and "Points to be Remembered"—terse, crisp reminders—stand out as particularly practical. Just the book for those who have the *home-care* of the sick.

Lewis' Anatomy and Physiology **Second Edition**

ANATOMY AND PHYSIOLOGY FOR NURSES. By LEROY LEWIS, M.D., formerly Surgeon to and Lecturer on Anatomy and Physiology for Nurses at the Lewis Hospital, Bay City, Michigan. 12mo of 375 pages, with 150 illustrations. Cloth, $1.75 net.

A demand for such a work as this, *treating the subjects from the nurse's point of view* has long existed. Dr. Lewis has based the plan and scope of this work on the methods employed by him in teaching these branches, making the text unusually simple and clear.

"It is not in any sense rudimentary, but comprehensive in its treatment of the subjects in hand. The application of the knowledge of anatomy in the care of the patient is emphasized."—*The Nurses Journal of the Pacific Coast.*

Friedenwald and Ruhräh's Dietetics **Second Edition**

DIETETICS FOR NURSES. By JULIUS FRIEDENWALD, M. D., Professor of Diseases of the Stomach, and JOHN RUHRÄH, M. D., Professor of Diseases of Children, College of Physicians and Surgeons, Baltimore. 12mo volume of 395 pages. Cloth, $1.50 net.

This work has been prepared to meet the needs of the nurse, both in the training school and after graduation. It aims to give the essentials of dietetics, considering briefly the physiology of digestion and the various classes of foods and the part they play in nutrition.

"It is exactly the book for which nurses and others have long and vainly sought. A simple manual of dietetics, which does not turn into a cook-book at the end of the first or second chapter."—*American Journal of Nursing.*

Macfarlane's Gynecology for Nurses

New (2d) Edition

A REFERENCE HAND-BOOK OF GYNECOLOGY FOR NURSES. By CATHARINE MACFARLANE, M. D., Gynecologist to the Woman's Hospital of Philadelphia. 16mo of 156 pages, with 70 illustrations. Flexible leather, $1.25 net.

"This is a nut-shell book, and the flexible leather covers are full of meat."—*Dietetic and Hygienic Gazette.*

Galbraith's Personal Hygiene and Physical Training for Women

PERSONAL HYGIENE AND PHYSICAL TRAINING FOR WOMEN. By ANNA M. GALBRAITH, M.D., Fellow New York Academy of Medicine. 12mo of 371 pages, with original illustrations. Cloth, $2.00 net.

Dr. Galbraith's book is just what has long been needed—a simple manual of hygiene and physical training along scientific lines.

De Lee's Obstetrics for Nurses

Just Ready
New (4th) Edition

OBSTETRICS FOR NURSES. By JOSEPH B. DE LEE, M. D., Professor of Obstetrics in the Northwestern University Medical School. 12mo volume of 507 pages, fully illustrated. Cloth, $2.50 net.

"It is far and away the best that has come to my notice, and I shall take great pleasure in recommending it to my nurses and students as well."—J. CLIFTON EDGAR, M. D., *Cornell Medical School, N. Y.*

Davis' Obstetric Nursing

Just Issued
New (4th) Edition

OBSTETRIC AND GYNECOLOGIC NURSING. By EDWARD P. DAVIS, A. M., M. D., Professor of Obstetrics, Jefferson Medical College and Philadelphia Polyclinic. 12mo of 480 pages, illustrated. Buckram, $1.75 net.

"Not only nurses, but even newly qualified medical men, would learn a great deal by a perusal of this book. It is written in a clear and pleasant style, and is a work we can recommend."—*The Lancet, London.*

Beck's Hand-Book for Nurses

New (3d) Edition

A REFERENCE HAND-BOOK FOR NURSES. By AMANDA K. BECK, of Chicago, Ill. 32mo of 230 pages. Flexible leather, $1.25 net.

This little book contains information upon every question that comes to a nurse in her daily work, and embraces all the information that she requires to carry out any directions given by the physician.

"Must be regarded as an extremely useful book, not only for nurses, but for physicians."—*Boston Medical and Surgical Journal.*

Stoney's Materia Medica for Nurses

Practical Materia Medica for Nurses, with an Appendix containing Poisons and their Antidotes, with Poison-Emergencies; Mineral Waters; Weights and Measures, etc. By EMILY M. A. STONEY, formerly at the Carney Hospital, South Boston, Mass. 12mo, 300 pages. $1.50 net.

THE NEW (3d) EDITION

In this work the consideration of the drugs includes their names, their sources and composition, their various preparations, physiologic actions, directions for handling and administering, and the symptoms and treatment of poisoning.

Journal of the American Medical Association

"So far as we can see, it contains everything that a nurse ought to know in regard to drugs. As a reference-book for nurses it will without question be very useful."

Bolduan and Grund's Bacteriology for Nurses

Just Ready

APPLIED BACTERIOLOGY FOR NURSES. By CHARLES F. BOLDUAN, M. D., Assistant to the General Medical Officer, and MARIE GRUND, M. D., Bacteriologist, Research Laboratory, Department of Health, New York City. 12mo of 155 pages, illustrated. Cloth, $1.25 net.

We were fortunate in getting these practical physicians to write this work. It gives particular emphasis to the immediate *application* of bacteriology to nursing, only the really practical being included. A study of all the modes of infection transmission is presented. At the end of each chapter are suggestions for practical demonstration.

Bohm and Painter's Massage

Just Ready

MASSAGE. By MAX BOHM, M. D., of Berlin, Germany. Edited, with an Introduction, by CHARLES F. PAINTER, M. D., Professor of Orthopedic Surgery at Tufts College Medical School, Boston. Octavo of 91 pages, with 70 *practical* illustrations. Cloth, $1.75 net.

Manhattan Hospital Eye, Ear, Nose, Throat Nursing

NURSING IN DISEASES OF THE EYE, EAR, NOSE, AND THROAT. By the Committee on Nurses of the Manhattan Eye, Ear, and Throat Hospital. 12mo of 260 pages, illustrated. Cloth, $1.50 net.

Paul's Fever Nursing

New (2d) Edition

NURSING IN THE ACUTE INFECTIOUS FEVERS. By GEORGE P. PAUL, M. D., formerly Assistant Visiting Physician to the Samaritan Hospital, Troy, N. Y. 12mo of 246 pages. Cloth, $1.00 net.

Dr. Paul has taken great pains in the presentation of the care and management of each fever. The book treats of fevers in general, then each fever is discussed individually, and the latter part of the book deals with practical procedures and valuable information.

"The book is an excellent one and will be of value to those for whom it is intended. It is well arranged, the text is clear and full, and the illustrations are good."—*The London Lancet.*

Paul's Materia Medica for Nurses

New (2d) Edition

MATERIA MEDICA FOR NURSES. By GEORGE P. PAUL, M.D., formerly Assistant Visiting Physician to the Samaritan Hospital, Troy. 12mo of 240 pages. Cloth, $1.50 net.

Dr. Paul arranges the physiologic actions of the drugs according to the action of the drug and not the organ acted upon. An important section is that on pretoxic signs, giving the warnings of the full action or the beginning toxic effects of the drug, which, if heeded, may prevent many cases of drug poisoning. The nurse should know these signs.

"This volume will be of real help to nurses: the material is well selected and well arranged, and the book is as readable as it is useful."—*The Medical Record.*

Pyle's Personal Hygiene

The New (5th) Edition

A MANUAL OF PERSONAL HYGIENE: Proper Living upon a Physiologic Basis. By Eminent Specialists. Edited by WALTER L. PYLE, A.M., M.D., Assistant Surgeon to Wills Eye Hospital, Philadelphia. Octavo volume of 515 pages, fully illustrated. Cloth, $1.50 net.

To this new edition there have been added, and fully illustrated, chapters on Domestic Hygiene and Home Gymnastics, besides an appendix containing methods of Hydrotherapy, Mechanotherapy, and First Aid Measures. There is also a Glossary of the medical terms used.

"The work has been excellently done, there is no undue repetition, and the writers have succeeded unusually well in presenting facts of practical significance based on sound knowledge."—*Boston Medical and Surgical Journal.*

Galbraith's Four Epochs of Woman's Life

Second Edition

THE FOUR EPOCHS OF WOMAN'S LIFE. By ANNA M. GALBRAITH, M.D. With an Introductory Note by JOHN H. MUSSER, M.D., University of Pennsylvania. 12mo of 247 pages. Cloth, $1.50 net.

"We do not as a rule care for medical books written for the instruction of the public; but we must admit that the advice in Dr. Galbraith's work is in the main wise and wholesome."—*Birmingham Medical Review, England.*

Spratling on Epilepsy

EPILEPSY AND ITS TREATMENT. By WILLIAM P. SPRATLING, M. D., formerly Professor of Physiology and Nervous Diseases, College of Physicians and Surgeons, Baltimore. Octavo of 522 pages, fully illustrated. Cloth, $4.00 net.

Peterson *and* Haines' Legal Medicine & Toxicology

A Text-Book of Legal Medicine and Toxicology. Edited by FREDERICK PETERSON, M. D., Professor of Psychiatry in the Columbia University (College of Physicians and Surgeons), New York; and WALTER S. HAINES, M. D., Professor of Chemistry, Pharmacy, and Toxicology, Rush Medical College, in affiliation with the University of Chicago. Two imperial octavo volumes of about 750 pages each, fully illustrated. Per volume: Cloth, $5.00 net; Sheep or Half Morocco, $6.50 net. *Sold by Subscription.*

TWO VOLUMES

The object of the present work is to give to the medical and legal professions a comprehensive survey of forensic medicine and toxicology in moderate compass. An interesting and important chapter is that on "The Destruction and Attempted Destruction of the Human Body by Fire and Chemicals." A chapter not usually found in works on legal medicine is that on "The Medicolegal Relations of the X-Rays."

Columbia Law Review

"For practitioners in criminal law and for those in medicine who are called upon to give court testimony in all its various forms . . . it is extremely valuable."

Fiske's Human Body

Structure and Functions of the Body. By ANNETTE FISKE, A.M., Graduate of the Waltham Training School for Nurses. 12mo of 221 pages, illustrated. Cloth, $1.25 net.

PRESENTED IN A NEW WAY

The way in which this book presents anatomy and physiology is a departure from the usual method—a departure, however, of a very practical kind. Miss Fiske has woven the physiology in with the anatomy, thus making her work a most readable one. It is an extremely practical book—one that can be readily understood.

Draper's Legal Medicine

A TEXT-BOOK OF LEGAL MEDICINE. By FRANK WINTHROP DRAPER, A. M., M. D., late Professor of Legal Medicine in Harvard University. Octavo of 573 pages, illustrated. Cloth, $4.00 net.

Golebiewski and Bailey's Accident Diseases

ATLAS AND EPITOME OF DISEASES CAUSED BY ACCIDENTS. By DR. ED. GOLEBIEWSKI, of Berlin. Edited, with additions, by PEARCE BAILEY, M. D., Consulting Neurologist to St. Luke's Hospital, New York. With 71 colored illustrations on 40 plates, 143 text-illustrations, and 549 pages of text. Cloth, $4.00 net. *In Saunders' Hand-Atlas Series.*

Chapman's Medical Jurisprudence — Third Edition

MEDICAL JURISPRUDENCE, INSANITY, AND TOXICOLOGY. By HENRY C. CHAPMAN, M. D., late Professor of Institutes of Medicine and Medical Jurisprudence in Jefferson Medical College, Philadelphia. 12mo of 329 pages, fully illustrated. Cloth, $1.75 net.

Hofmann and Peterson's Legal Medicine — In Saunders' Hand-Atlases

ATLAS OF LEGAL MEDICINE. By DR. E. VON HOFMANN, of Vienna. Edited by FREDERICK PETERSON, M. D., Professor of Psychiatry in the College of Physicians and Surgeons, New York. With 120 colored figures on 56 plates and 193 half-tone illustrations. Cloth, $3.50 net.

Jakob and Fisher's Nervous System — Saunders' Hand-Atlases

ATLAS AND EPITOME OF THE NERVOUS SYSTEM AND ITS DISEASES. By PROFESSOR DR. CHR. JAKOB, of Erlangen. Edited, with additions, by EDWARD D. FISHER, M. D., University and Bellevue Hospital Medical College, New York. With 83 plates and copious text. Cloth, $3.50 net.

Crothers' Morphinism and Narcomania

MORPHINISM AND NARCOMANIA. By T. D. CROTHERS, M. D. 12mo of 351 pages. Cloth, $2.00 net.

Abbott's Transmissible Diseases — Second Edition

THE HYGIENE OF TRANSMISSIBLE DISEASES: Their Causes, Modes of Dissemination, and Methods of Prevention. By A. C. ABBOTT, M. D., Professor of Hygiene and Bacteriology, University of Pennsylvania. Octavo of 351 pages, illustrated. Cloth, $2.50 net.

American Pocket Dictionary

Just Ready
New (8th) Edition

AMERICAN POCKET MEDICAL DICTIONARY. Edited by W. A. NEWMAN DORLAND, M.D. Containing the pronunciation and definition of the principal words used in medicine and kindred sciences, with 64 extensive tables. With 677 pages. Flexible leather, with gold edges, $1.00 net; with patent thumb index, $1.25 net.

Morrow's Immediate Care of Injured

Second Edition

IMMEDIATE CARE OF THE INJURED. By ALBERT S. MORROW, M. D., Attending Surgeon to the New York City Hospital for the Aged and Infirm. Octavo of 360 pages, with 242 illustrations. Cloth, $2.50 net.

Dr. Morrow's book on emergency procedures is written in a definite and decisive style, the reader being told just what to do in every emergency. It is a practical book for every day use, and the large number of excellent illustrations can not but make the treatment to be pursued in any case clear and intelligible. Physicians and nurses will find it indispensible.

Starr's Diets for Infants and Children

DIETS FOR INFANTS AND CHILDREN IN HEALTH AND IN DISEASE. By LOUIS STARR, M. D., Consulting Pediatrist to the Maternity Hospital, Philadelphia. 230 blanks (pocket-book size). Bound in flexible Morocco, $1.25 net.

Grafstrom's Mechano-Therapy

2d Revised Edition

A TEXT-BOOK OF MECHANO-THERAPY (Massage and Medical Gymnastics). By AXEL V. GRAFSTROM, B. Sc., M.D., Attending Physician to the Gustavus Adolphus Orphanage, Jamestown, New York. 12mo, 200 pages, illustrated. Cloth, $1.25 net.

Shaw on Nervous Diseases and Insanity

Fourth Edition, Revised

ESSENTIALS OF NERVOUS DISEASES AND INSANITY: their Symptoms and Treatment. A Manual for Students and Practitioners. By the late JOHN C. SHAW, M. D., Clinical Professor of Diseases of the Mind and Nervous System, Long Island College Hospital, New York. 12mo of 204 pages, illustrated. Cloth, $1.00 net. *In Saunders' Question-Compend Series.*

Powell's Diseases of Children

3d Edition, Revised

ESSENTIALS OF THE DISEASES OF CHILDREN. By WILLIAM M. POWELL, M. D. Revised by ALFRED HAND, JR., A. B., M. D., Dispensary Physician and Pathologist to the Children's Hospital, Philadelphia. 12mo volume of 259 pages. Cloth, $1.00 net. *In Saunders' Question-Compend Series.*

RETURN TO the circulation desk of any
University of California Library
or to the
NORTHERN REGIONAL LIBRARY FACILITY
Bldg. 400, Richmond Field Station
University of California
Richmond, CA 94804-4698

ALL BOOKS MAY BE RECALLED AFTER 7 DAYS
2-month loans may be renewed by calling
(415) 642-6233
1-year loans may be recharged by bringing books
to NRLF
Renewals and recharges may be made 4 days
prior to due date

DUE AS STAMPED BELOW

LIBRARY USE JAN 15 '87

YB 65626

Saunders' Compends

ıstrations.
IATHEWS,

ı edition.

G, AND
ons. By

AL DIS-
tion, fully

ed., illus-

ed. By

S-COHEN,

Illustrated.

ustrations

SANITY.
Revised

d., illus-

ons. By

e, Phila.